Comparison of Optical Coherence Tomography Angiography Findings between Healthy Children and Children with Type 1 Diabetes Mellitus and Autoimmune Thyroiditis

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Abstract

Aim: This study aimed to compare the development of early diabetic retinopathy (DR) findings, a microvascular complication, between patients with type 1 DM and autoimmune thyroiditis (AT) (group 1), patients with isolated type 1 diabetes mellitus (DM) (group 2), and healthy controls (group 3), who were matched for age, sex, number, and body mass index for comparison.

Methods: In this prospective observational study, individuals aged 10–20 years were included, and patients in groups 1 and 2 were followed up for ≥5 years. None of them developed clinical DR during the follow-up period. Optical coherence tomography angiography (OCTA) was used to evaluate the foveal avascular zone (FAZ) and parafoveal vascular density (PVD) for the development of early DR among the groups. OCTA findings were compared between patients and healthy controls. Obtained data were analyzed using IBM SPSS Statistics for Windows, version 25.0.

Results: The mean FAZ and PVD were significantly different among the three groups (FAZ, p = 0.016; PVD, p = 0.006). The mean FAZ was higher in groups 1 and 2 than in group 3 (p = 0.059 and 0.007, respectively). No significant difference was found between groups 1 and 2 in terms of the mean FAZ and PVD (p = 0.832 and 0.653, respectively). The mean glycated hemoglobin (HbA1c) level was significantly correlated with FAZ and PVD (FAZ: r = 0.496, p < 0.001; PVD: r = 0.36, p = 0.001).

Conclusion: In patients with type 1 DM who did not develop clinical DR, OCTA findings revealed an increase in FAZ, which was associated with higher HbA1c levels. The mean PVD was significantly lower in the group with coexisting AT and type 1 DM than in the control group. These results suggest that the coexistence of AT and type 1 DM can contribute to the development of microvascular complications. Thus, studies with larger patient series are required.

Keywords: autoimmune thyroiditis, diabetic retinopathy, foveal avascular zone, optical coherence tomography angiography, parafoveal vessel density, type 1 diabetes mellitus

Introduction

Owing to the prevalence, associated complications, and cost of diabetes, it is recognized as the fastest growing global health issue. In 2021, over 1.2 million children and adolescents had type 1 diabetes mellitus (T1DM) (1). In the first nationwide report on diabetes in Turkey, the prevalence of T1DM was 0.75/1000 from January 2011 to December 2013, and the mean of the age of patients at diagnosis was 10.6 ± 4.6 years (2). In another study of children aged <18 years from 2013 to 2015 in Northwest Turkey, 1773 patients (588, 592, and 593 in 2013, 2014, and 2015, respectively) were diagnosed with T1DM. The crude mean incidence was 8.99/100,000 (3). Given the cruel face of diabetes, the life expectancy of patients with diabetes is reduced by 10 years–6. In the majority of the developed societies, DM is identified as a leading cause of blindness, renal failure, and lower limb amputation (4–6). Moreover, DM-related complications progress rapidly. Every day, 225 people undergo foot amputation, 120 people undergo dialysis, and 55 people lose their vision. Moreover, diabetic retinopathy (DR) is probably the most characteristic, easily identifiable, and treatable complication of DM; however, it remains an important cause of vision loss in developed countries (7). The prevalence of DR in patients with T1DM ranges from 10.8% to 60.0% in clinic-based populations and from 14.5% to 79.0% in population-based studies (1).

Indirect ophthalmoscopy is known as the primary approach for screening DR, and it has high diagnostic accuracy (8). Currently, fluorescein angiography considered the gold standard for the definitive diagnosis and grading of DR, but it needs an intravenous dye injection, which can cause nausea, vomiting, and hypersensitivity (9). Optical coherence tomography angiography (OCTA) is a quick noninvasive procedure that may be performed without using a dye. These properties of OCTA can be quite beneficial, particularly for the pediatric population. Moreover, it is a potentially useful screening and follow-up tool for children with T1DM. Most OCTA studies have focused on adult patients with DR. In these studies, the mean foveal avascular zone (FAZ) in patients with DR was significantly greater than that of healthy controls. In addition, patients with DR had significantly lower parafoveal vascular density (PVD) (10–12). Several studies examined children with T1DM to assess FAZ and PVD; however, no consensus has been achieved to date (13–15). Thus, further studies are required to reach clear conclusions on this topic. Moreover, novel parameters such as FAZ and PVD, which can be subjected to automatic quantitative analysis using OCTA software, may help analyze early-onset T1DM without retinopathy screening and disease follow-up.

This study aimed to assess the retinal vessel density and FAZ area, as assessed using OCTA, in patients with isolated T1DM (group 1) and those with T1DM and autoimmune thyroiditis (AT) (group 2). Moreover, it aimed to compare potential pathologic early changes in this population with those in healthy age-matched controls (group 3).

Methods

Patients with isolated T1DM (group 1), patients with both T1DM and AT (group 2), and healthy volunteers (group 3) were included in the present study. Furthermore, participants in these three groups were matched for age, race, sex, number (n = 32), and body mass index. Patients with DM were consecutively enrolled from the outpatient clinic of the Department of Pediatric Endocrinology, İzmir Dr. Behçet Uz
Children’s Training and Research Hospital, for routine follow-up. Inclusion criteria for patients with DM were age of 10–20 years, diabetes duration of >5 years, normotonia, body mass index less than the age- and sex-specific 95th percentile, absence of chronic diseases other than T1DM or AT, no other autoimmune diseases, and no history of smoking. None of the patients with DM took any medication other than insulin or levothyroxine on a daily basis. Moreover, patients with DM were selected based on a similar ratio for poor glycemic control, and they received the same dose of insulin. All patients with DM were undergoing conventional insulin therapy and had T1DM without complications such as nephropathy or neuropathy. No severe hypoglycemic events that could cause coma and/or seizure were reported in patients with T1DM. Insulin pump or hybrid closed-loop therapy was not used for patients with T1DM. There were no differences between participants and all eligible diabetes hospital populations of the same age in terms of the clinical characteristics. The control group included healthy individuals matched for age, race, sex, number (n = 32), and body mass index.

The healthy controls included in the study were friends of the participants with DM and the participants treated at Izmir Dr. Behcet Uz Children’s Training and Research Hospital. Written informed consent was obtained from the legal guardians of the participants. The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Joint Commission on Ethics of Izmir Dr. Behcet Uz Children’s Training and Research Hospital.

Blood pressure (BP) measurements

BP was measured in a quiet room during the regular 3-month follow-up visits. Notably, BP measurements were obtained using a conventional oscillatory measurement system positioned at the right-upper arm (DINAMAP; GE Healthcare, Munich, Germany). The cuff size was selected according to the patient’s arm circumference, with the cuff bladder covering ≥40% and ≤100% of the arm circumference. Standard deviation (SD) values were calculated by adopting normal values from the study in the relevant literature (16).

Laboratory methods

Blood samples were obtained at 8:00 a.m. after an overnight fasting for ≤12 h during the patients’ follow-up visit. Levels of fasting glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were measured using the standard laboratory methods. Each sample was processed immediately after the patient’s visit with a maximum delay of 1 h. Moreover, blood samples were obtained on the day of ocular examination in the DM group for measuring retinal neovascularization and peripapillary atrophy. Blood glucose and 1-year mean glycated hemoglobin (HbA1c) levels. Further, the duration of T1DM and levels of HbA1c were examined. The blood samples for FAZA and OCTA measurement were analyzed using the designated method of National Glycohemoglobin Standardization Program.

OCTA and ophthalmological evaluation

Slit-lamp examination and indirect ophthalmoscopy were used to examine the clinical symptoms of DR in all patients. Patients with retinopathy findings, any ocular diseases, prior ocular surgery, myopia or hypermetropia higher than four diopters (D) were excluded from the study. Avanti RTVue XR AngioVue (Optovue) was used to perform OCTA, and 3 3 × 3 mm pictures of the retina centered on the fovea were acquired. The AngioVue program was used to calculate the area of FAZ. In particular, the device software used in this study produced a vascular image of the retinal layer to a depth of 10 μm, from the inner limiting membrane to the outer plexiform layer. The FAZ boundary was determined using this method. For assessing the density of blood vessels in a 300-μm wide ring encircling FAZ (Figure 1).

Statistical Analysis

Statistical analyses were performed using the IBM SPSS Statistics for Windows Version 25 (IBM Corp., Armonk, NY, USA). The mean, SD, or percentage values were used to express the obtained data. Subsequently, a separate multivariate linear regression model, including significant variables identified from univariate analyses, was used to determine the factors that independently explained a significant difference (P < 0.05) between dependent variables. Quantitative data were expressed as median (minimum–maximum). The Kruskal–Wallis and post hoc Mann–Whitney U tests were used to compare values among the three groups. Spearman’s rank correlation coefficient tests were used for the univariate analysis. A perfect correlation was considered to be indicated by a Spearman’s rank correlation coefficient of 1, whereas a negative correlation was indicated by a Spearman’s rank correlation coefficient of –1. A p-value of < 0.05 was considered significant.

Results

The characteristics of the study groups are presented in Table 1. The mean DM duration was 8 (5.6–10.2) years. In the DM group, the 1-year mean HbA1c level was 9.10% (6.3%–15.0%), with reference range 4.7%–5.7%. The median daily insulin dose was 0.96 (0.80–1.10) IU/kg. No significant differences in age, sex, weight, and body mass index were found among the three groups. Groups 1 (172 [148–188] mg/dL) and 2 (168 [149–195] mg/dL) had higher mean total cholesterol levels than group 3 (150 [132–172] mg/dL; p = 0.011), whereas no differences were found in terms of serum triglyceride, LDL, and HDL cholesterol levels among the three groups. Although no differences were found in the systolic BP among the study groups, groups 1 (60.2 ± 7.69 mmHg) and 2 (65.33 ± 8.06 mmHg) had a higher diastolic BP than the control group (p = 0.020) (Table 1).

In all subjects, the BCVA was 20/20. The mean FAZ and PVD were significantly different among the groups (p = 0.016 and 0.006, respectively). The mean FAZ was higher in groups 1 and 2 than in group 3 (p = 0.013 and 0.119, respectively) (Figure 2). The mean PVD was lower in groups 1 and 2 than in group 3 (p = 0.059 and 0.007, respectively) (Figure 3). No significant difference was found between groups 1 and 2 in terms of the mean FAZ and PVD (p = 0.832 and 0.653, respectively). The correlations between the retinal FAZ and PVD and FAZ are shown separately for groups 1–3 in Figure 4 and 5. A significant correlation was found between mean HbA1c levels and FAZ and PVD (FAZ: r = 0.496, p < 0.001; PVD: r = −0.36, p = 0.001), whereas no correlation was found between other parameters and FAZ and PVD. Moreover, no correlation was found between thyroid-stimulating hormone (TSH) levels and FAZ and PVD. According to multivariate linear regression analyses, the correlation between FAZ and HbA1c has persisted (p = 0.001). There was also a correlation between PVD and HbA1c in multivariate analyses (p = 0.001).

Discussion

Many researchers have suggested that early DR in T1DM is associated with an increased risk of poor glucose control, high HbA1c level, hypertension, and dyslipidemia (17–20). However, the effects of T1DM and AT on capillary endothelial structure and retinal microcirculation remain unclear. Based on the results of our study, we suggest that the coexistence of AT and T1DM is associated with detrimental effects on the capillary endothelial function because of impaired glucose control in patients with T1DM. To the best of our knowledge, this is the first study to assess the potential early pathologic changes of DR in children with isolated T1DM, children with AT and T1DM, and children with T1DM and AT (17–20).}

[The rest of the discussion would continue here, discussing the implications of the findings, the limitations of the study, and future research directions.]
chlororetinopathy and hypothyroidism, but the effect of the mean arterial pressure and blood glucose abnormalities on capillary endothelial dysfunction were not considered by them (22). Moreover, they did not consider lipid abnormalities and peroxidation caused by hypothyroidism. Wysocka-Mincewicz et al. did not find a relationship between FAZ and TSH (21). Similarly, our results did not reveal a correlation among FAZ, PVD, and TSH. One et al. compared the FAZ of 29 children with T1DM to that of 24 healthy children and found that FAZ was greater in patients with T1DM, but there was no difference in terms of PVD (14). Golpishkawa et al. compared the OCTA parameters of 94 children with T1DM to those of 36 healthy children and found no difference between the two groups. However, elevated HbA1c levels were found to be correlated with reduced parafoveal superficial vessel density and parafoveal thickness (23). Inanc et al. compared the onset of DR between 60 children with T1DM and 57 age-matched controls, and they found a greater FAZ in children with T1DM (13). In the present study, the mean FAZ and PVD differed between children with DM and healthy children. Moreover, we found that DR changes started early in children with DM and were related to poor glucose control. These findings suggested that AT had no effect on the development of DR in cases of no lipid abnormalities and peroxidation. Hence, we hypothesized that early DR signs may develop independently of AT in children with T1DM and AT.

HbA1c levels in patients with high indicate high blood glucose levels during the day. Further, high serum glucose levels are associated with microvascular complications such as DR (17–20). Notably, even 1% reduction in HbA1c levels in patients with DM leads to a 32% reduction in microvascular complications. The increased plasma pathway, increased advanced glycation end-product (AGE) formation, activation of protein kinase C (PKC) isoforms, and increased hexosamine pathway pathway, as an adaptation mechanism (24,25). In patients with DM, high serum glucose levels increase glycosylation and AGE formation. Further, in the vascular endothelium of all organs, an increase in AGE levels causes binding to receptors for AGES (RAGES). As RAGES are present in eye blood vessels, they bind to the capillary endothelium, which further leads to the development of retinopathy. In patients with hyperglycemia, serum glucose must find a pathway to be metabolized, and it activates the beta and gamma isoforms of PKC via the diacylglycerol (DAG) pathway. Increased PKC levels activate NADPH oxidases and increase reactive oxygen radicals. Further, an excessive increase in reactive oxygen radicals leads to complications. Moreover, the increased glucose is metabolized via the polyol pathway by aldose reductase. Notably, the activation of the polyol pathway increases the use of hydrogen. Hydrogen is required for the production of nitric oxide (NO) from arginine. Although hydrogen is not directly involved in the synthesis of NO, it is involved in the polyol pathway as a compensatory mechanism; this decreases NO synthesis. Vasoconstriction and ischemia caused by the reduction of NO and the activation of DAG PKC cause provocations in the bloodstream (24,25). Moreover, increasing vascular endothelium hypoxia increases growth factors, such as vascular endothelial growth factor. Furthermore, it leads to an increase in nuclear factor kappa B, which suppresses inflammatory genes and promotes irreversible acceleration. This further leads to vasoconstriction, oxidation, and inflammation. Given that DM is an endothelial disease, defense against oxidation is poor patients with DM, and signs of early DR are attributed to proliferation, as observed in our cases. We found a relationship between HbA1c levels and FAZ and PVD; however, we did not find a relationship between thyroid function tests and FAZ and PVD.

This study has some limitations. First, this was a single-center study with a relatively small sample, and our post-hoc analysis included children with T1DM and AT. Second, the study was cross-sectional and could not describe the long-term effects of the disease and its treatment. However, we believe that the study is meaningful as it provides data on the retinal microcirculation of OCTA parameters in the analyzed subgroup. Third, all OCTA parameters examined in the study were influenced by age. Therefore, the results should be interpreted taking into account the potential effect of age on blood glucose control on the DR changes in children with AT and T1DM. Quantitative assessments in OCTA may be impacted by axial length. The fact that the children’s axial length was not assessed is one of the study’s shortcomings. However, it is feasible to assume that when groups of similar ages and without distinct refractive errors are compared, the difference between the axial lengths of the groups will be limited.

Conclusion

Our data suggest that the coexistence of AT and T1DM in children with clinically detectable DR leads to impaired retinal microcirculation and FAZ margin irregularities, similar to children with T1DM matched for age, race, sex, number, body mass index, BP, and plasma lipid levels. Impairment in retinal microcirculation is signs of diabetic retinopathy can develop independently of AT in children with T1DM and AT. Further studies are needed to evaluate the role of OCTA in early disease detection and treatment counseling in children with both AT and T1DM. Further, early monitoring of microvascular risk factors is required.

References

<table>
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<tr>
<th></th>
<th>AT + T1DM (n=32)</th>
<th>T1DM (n=32)</th>
<th>Healthy Children (n=32)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>15.6 (14.0 – 18.7)</td>
<td>15.4 (13.7 – 17.2)</td>
<td>15.3 (14.2 – 18.2)</td>
<td>0.851</td>
</tr>
<tr>
<td>Male (n, %)</td>
<td>9 (28.1%)</td>
<td>9 (28.1%)</td>
<td>9 (28.1%)</td>
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<tr>
<td>Weight SDS</td>
<td>0.15 (-1.1 – 1.2)</td>
<td>0.21 (-0.7 – 0.8)</td>
<td>0.22 (-1.0 – 1.2)</td>
<td>0.975</td>
</tr>
<tr>
<td>Height SDS</td>
<td>-0.47 (-1.0 – 0.1)a</td>
<td>-0.09 (-0.6 – 0.6)</td>
<td>0.22 (-0.2 – 1.1)b</td>
<td>0.004</td>
</tr>
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<td>BMI SDS</td>
<td>0.41 (-0.6 – 1.3)</td>
<td>0.46 (-0.3 – 0.8)</td>
<td>-0.21 (-1.1 – 1.0)</td>
<td>0.289</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120 (115 – 128)</td>
<td>120 (111,8 – 127.8)</td>
<td>119.5 (100.8 – 128.8)</td>
<td>0.73</td>
</tr>
<tr>
<td>DBP(mmHg)</td>
<td>80 (74.3 – 87)b</td>
<td>79.5 (65.8 – 85)a</td>
<td>69 (66.3 – 75.8)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T1DM duration (years)</td>
<td>8.1 (5.9 – 9.7)</td>
<td>8.0 (5.4 – 11)</td>
<td>-</td>
<td>0.941*</td>
</tr>
<tr>
<td>Insulin dose (IU/kg/day)</td>
<td>0.9 (0.8 – 1.1)</td>
<td>1.0 (0.9 – 1.2)</td>
<td>-</td>
<td>0.434*</td>
</tr>
<tr>
<td>PGC (n, %)</td>
<td>14 (43.8%)</td>
<td>17 (53.1%)</td>
<td>-</td>
<td>0.617</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>217 (155 – 289)b</td>
<td>263 (177 – 301)a</td>
<td>89 (84 – 95)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean HbA1c (%)</td>
<td>9.4 (6.3 – 15.2)b</td>
<td>9.05 (6.8 – 12.8)b</td>
<td>5 (4.9 – 5.4)b</td>
<td>&lt;0.001</td>
</tr>
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<td>Triglyceride (mg/dl)</td>
<td>92 (64 – 130)</td>
<td>88 (64 – 110)</td>
<td>85 (62 – 99)</td>
<td>0.315</td>
</tr>
<tr>
<td>HDL(mg/dl)</td>
<td>57 (49 – 64)</td>
<td>61 (53 – 69)</td>
<td>56 (47 – 63)</td>
<td>0.122</td>
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<tr>
<td>LDL(mg/dl)</td>
<td>89 (78 – 105)</td>
<td>89 (68 – 102)</td>
<td>84 (65 – 92)</td>
<td>0.064</td>
</tr>
<tr>
<td>TC(mg/dl)</td>
<td>168 (149 – 195)b</td>
<td>172 (148 – 168)a</td>
<td>150 (132 – 172)b</td>
<td>0.011</td>
</tr>
<tr>
<td>fT4 (ng/dL)</td>
<td>1.24 (1.13 – 1.35)</td>
<td>1.23 (1.14 – 1.36)</td>
<td>1.27 (1.17 – 1.38)</td>
<td>0.621</td>
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<tr>
<td>TSH (μIU/mL)</td>
<td>3.13 (2.20 – 4.58)b</td>
<td>2.84 (1.79 – 3.46)</td>
<td>2.14 (1.57 – 2.98)b</td>
<td>0.004</td>
</tr>
<tr>
<td>Anti-TG (IU/ml)</td>
<td>68 (31 – 149)b</td>
<td>16 (14 – 20)a</td>
<td>17 (15 – 21)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-TPO (IU/ml)</td>
<td>144 (23 – 271)b</td>
<td>11 (10 – 13)a</td>
<td>11 (10 – 13)b</td>
<td>&lt;0.001</td>
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<td>FAZ</td>
<td>0.301 ± 0.03</td>
<td>0.303 ± 0.05</td>
<td>0.270 ± 0.03</td>
<td>0.006**</td>
</tr>
<tr>
<td>PVD</td>
<td>52.0 ± 3.2</td>
<td>52.9 ± 2.7</td>
<td>54.6 ± 1.2</td>
<td>0.016**</td>
</tr>
</tbody>
</table>

Variables are shown as median (interquantile range), p<0.05. *Mann-Whitney U test. **Post-hoc analysis p<0.0167

** This value of the results of the Kruskal-Wallis test indicates group differences, but does not indicate which groups are different.

AT: Antimicros thyroiditis, BMI: body mass index, SDS: Standart deviation score, SBP: systolic blood pressure, DBP: diastolic blood pressure, T1DM: type 1 diabetes mellitus, PGC: Poor glucose control, FBG: fasting blood glucose, HbA1c: glycated hemoglobin, HDL: high-density lipoprotein, LDL: low-density lipoprotein, TC: total cholesterol, fT4: free thyroxine, TSH: thyroid stimulating hormone, anti-TG: thyroglobulin antibody, Anti-TPO: thyroid peroxidase antibody
Figure 1. Examples of OCTA images with predicted foveal avascular zone area and parafoveal vessel density (A) a 14-year-old healthy boy's right eye with a foveal avascular zone area of 0.254 mm² and a parafoveal vessel density of 55.06. (B) a 15-year-old boy with T1DM has a foveal avascular zone area of 0.456 mm² and a parafoveal vessel density of 57.83.
Figure 2. The average foveal avascular zone areas of the three groups

Figure 3. The average parafoveal vessel density of the three groups
Figure 4. Correlation between mean HbA1C and FAZ.
Figure 5. Correlation between mean HbA1C and PVD