Influence of puberty on retinal microcirculation in children with type 1 diabetes without retinopathy using optical coherence tomography angiography

Marta Wysocka-Mincewicz[1], Joanna Gołębiewska[2,3],
Marta Baszyńska-Wilk[1] (iD), Andrzej Olechowski[2,4] (iD),
Aleksandra Byczyńska[1] and Mieczysław Szalecki[1,5]

Abstract

Background: This study aimed to assess the influence of pubertal status on the results of optical coherence tomography angiography (OCTA) in children with type 1 diabetes (T1D).

Methods: We enrolled 167 consecutive children with T1D. Retinal superficial capillary plexus (SCP) and deep capillary plexus (DCP) vessel density data underwent analysis. We divided the study population into three subgroups depending on the pubertal status.

Results: Analysis of the prepubertal and pubertal subgroups revealed statistically significant differences in foveal thickness (FT) ($p < 0.05$) and foveal SCP ($p < 0.02$). Analyzing subgroups of the prepubertal and postpubertal children, we observed statistically significant differences in FT ($p < 0.03$), whole SCP ($p < 0.02$), and foveal SCP ($p < 0.02$). Comparison of the pubertal and postpubertal subjects revealed differences in parafoveal DCP ($p < 0.003$). In the groups matched depending on diabetes duration, we observed differences between prepubertal, pubertal, and postpubertal children in FT, PFT, and parafoveal SCP and DCP.

Conclusion: Our data suggest that in a cohort of pubertal children with a short duration of diabetes, alterations in retinal vessel density occur early and progress during puberty.

Keywords

Type 1 diabetes, optical coherence tomography angiography, children, puberty

Introduction

Diabetic retinopathy (DR) is a sight–threatening complication of diabetes, usually asymptomatic in early stages. Most studies indicate that the first 5 years of life carries a relatively low risk of developing diabetes-related complications and pediatric diabetes retinopathy remains rare.[1,2] The main risk factors for the development of DR in children with type 1 diabetes (T1D) are the duration of the disease and the timing of puberty.[3–5] The Olsen study proved that the risk of DR increases twice in postpubertal years as compared to pre-pubertal years, which may be due to raised hemoglobin A1c levels and poorer metabolic control during puberty.[6] Therefore early detection of retinal abnormalities in these young patients is essential to prevent irreversible damage to the retina.

New imaging technologies may be useful in the early identification of retinal structural and functional changes before DR is clinically detectable. Optical coherence tomography angiography (OCTA) is a non-invasive tool

[1]Department of Endocrinology and Diabetology, Children's Memorial Health Institute, Warsaw, Poland
[2]Department of Ophthalmology, Children's Memorial Health Institute, Warsaw, Poland
[3]Faculty of Medicine, Lazarski University, Warsaw, Poland
[4]Ophthalmology Department, James Cook University Hospital, Middlesbrough, UK
[5]Collegium Medicum, Jan Kochanowski University, Kielce, Poland

Corresponding author:
Marta Wysocka-Mincewicz, Department of Endocrinology and Diabetology, Children's Memorial Health Institute, Al. Dzieci Polskich 20, Warsaw 04-730, Poland.
Email: m.wysocka@ipczd.pl

that enables a reproducible, quantitative assessment of the retinal microcirculation.[7] There are many reports assessing the retinal microcirculation in adult diabetes patients in the current literature, but only a few have focused on diabetes children.[8–11] This study aimed to assess the influence of pubertal status on OCTA results in children with T1D.

Material and methods

We performed an observational study on 167 consecutive children with T1D who met the inclusion criteria and agreed to participate in the study. The study was approved by the Bioethics Committee of Children's Memorial Health Institute in Warsaw and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from the patient's legal guardian and from patients >16 years old after explaining the nature of the non-invasive study.

The inclusion criteria were a diagnosis of T1D based on the International Society for Paediatric and Adolescent Diabetes (ISPAD) criteria and insulin treatment. Exclusion criteria were history of prematurity, other concomitant retinal pathologies, such as hereditary retinal dystrophies, vitreoretinal diseases, chorioretinal diseases, myopia, or hypermetropia (>6 diopters), or low quality of OCTA images. Both eyes' results were analyzed because of the influence of metabolic parameters and possible interocular differences. Metabolic control was measured by the current glycated hemoglobin A1c level (HbA1c) and the mean value for the whole diabetes duration (minimum four tests per year). Anthropometric data including age, weight, height, age at onset, and diabetes duration were recorded. The study population was divided into three subgroups depending on the pubertal status, based on the Tanner scale: prepubertal (Tanner scale 1), pubertal (if any signs of pubertum were observed, Tanner stage 2–4), and postpubertal (Tanner scale 5).[12] We performed additional analysis to eliminate possible influence of diabetes duration after matching groups dependently on this parameter.

Each patient underwent a complete ophthalmological examination, including best-corrected visual acuity (BVCA) assessment, slit-lamp biomicroscopy, fundus examination, and color fundus photography. Pupils were dilated with 1% tropicamide eye drops before the fundus examination and OCTA image collection. We performed OCTA using a commercially available RTVue XR Avanti (Version 2018.0.0.18) with AngioVue (Optovue, Fremont, CA, USA) with $3 \text{mm} \times 3 \text{mm}$ images of macula centred on the fovea. Each OCTA en face image contains 304×304 pixels created from the 304 vertical and the 304 horizontal B-scans. AngioVue automatically segments the area into four layers, including SCP, DCP, outer retinal layer, and choriocapillaris. The SCP en face image was segmental with an inner boundary at $3 \mu m$ beneath the internal limiting membrane and outer boundary set at $15 \mu m$, beneath the inner plexiform layer. In contrast, the deep capillary plexus en face image was segmented with an inner boundary $15 \mu m$ beneath the inner plexiform layer and an outer boundary at $70 \mu m$ beneath the inner plexiform layer. Vessel density was defined as the percentage of area occupied by vessel lumens after binary reconstruction of images. The SCP and DCP vascular density data were analyzed separately. For final analysis, the SCP and DCP whole, foveal and parafoveal data were taken. FT (μm) and PFT (μm) data were obtained from retinal maps, using the same device. We stabilized the head position with the standard chinrest and forehead support. Subjects were instructed to focus on an internal fixation target. After capturing three scans, we chose the best quality (with a signal strength index >60) for analysis. All measurements were performed at the same time of the day (between 9.00 and 11.00 AM) in all children to avoid the effect of diurnal variation of the results. Two trained OCTA readers reviewed all images independently to ensure correct segmentation and to identify low-quality scans, with motion artifacts or blurred images, where data were insufficient for proper analysis. We included data collected from each eye of patients into the independent analysis.

Statistical analysis

Data were described by mean and standard deviation values. Normal distribution was checked using the Shapiro-Wilk test. Differences between two groups in parameters with normal distribution were tested by *T*-Student test, or the *U* Mann Whitney test for non-normally distributed data, and between three subgroups by Kruskal-Wallis ANOVA by Ranks for independent groups. A level $p < 0.05$ was recognized as statistically significant. Statistical tests were carried out using TIBCO Software Inc. (2017) Statistica version 13 StatSoft Company.

Results

In total, we recruited 167 children (334 eyes) with T1D to the study. All children were Caucasian. Due to the poor quality of OCTA images, 26 eyes were excluded, and we included 308 in the final analysis. No child had any signs of diabetic retinopathy on fundus examination and colour fundus photography. Detailed data on the study population are summarized in Table 1.

Analysis of the prepubertal and pubertal subgroups revealed statistically significant differences in FT ($p < 0.05$), and foveal SCP ($p < 0.02$) (Figure 1).

When analyzing subgroups of the prepubertal and postpubertal children we observed statistically significant differences in FT ($p < 0.03$), whole SCP ($p < 0.02$) and foveal SCP ($p < 0.02$) (Figure 2).

Comparison of the pubertal and postpubertal revealed only differences in parafoveal DCP ($p < 0.003$) (Table 2) (Figure 3).

Table 1. The characteristics of the studied subgroups.

	Prepubertal group	Pubertal group	Postpubertal group	p Level
	$n=52$, girls$=24$, boys$=28$	$n=70$, girls$=32$, boys$=38$	$n=45$, girls$=26$, boys$=19$	
Age	8.37 ± 1.6	13.1 ± 1.5	17.2 ± 0.7	$p<0.00$
Age at the onset (years)	5.6 ± 2.3	8.7 ± 3.4	10.5 ± 4.3	$p<0.00$
Diabetes duration (years)	2.74 ± 2.0	4.38 ± 3.5	6.7 ± 4.4	$p<0.00$
Weight (kg)	30.6 ± 12.5	48.3 ± 11.3	67.8 ± 12.5	$p<0.00$
Height (cm)	133.5 ± 13.3	161.8 ± 11.2	172.25 ± 9.5	$p<0.00$
HbA1c current (%)	7.94 ± 2.1	8.3 ± 1.8	8.6 ± 1.4	$p<0.00$
HbA1c mean (%)	7.9 ± 1.9	8.06 ± 1.4	8.4 ± 1.3	$p<0.00$

HbA1c: glycated hemoglobin.

Table 2. Comparison of the prepubertal, pubertal, and postpubertal results.

	Prepubertal group	Pubertal group	p Level (prepubertal vs pubertal)	Postpubertal group	p Level (pubertal vs postpubertal)	p Level (prepubertal vs postpubertal)
FT (μm)	251.65 ± 19.1	256.6 ± 19.1	$p<0.05$	257.8 ± 18.8	$p=0.6$	$p<0.03$
PFT (μm)	317.4 ± 16.9	318.2 ± 17.1	$p=0.7$	318.97 ± 14.9	$p=0.7$	$p=0.5$
Whole SCP	51.85 ± 2.7	51.36 ± 3.0	$p=0.3$	50.75 ± 2.6	$p=0.2$	$p<0.02$
Foveal SCP	34.58 ± 5.35	32.61 ± 5.4	$p<0.02$	32.53 ± 4.5	$p=0.9$	$p<0.02$
Parafoveal SCP	53.15 ± 2.98	53.1 ± 3.37	$p=0.9$	52.24 ± 2.9	$p=0.9$	$p=0.07$
Whole DCP	58.1 ± 2.2	58.5 ± 2.2	$p=0.2$	57.97 ± 1.6	$p=0.07$	$p=0.07$
Foveal DCP	32.6 ± 6.7	32.3 ± 6.1	$p=0.8$	32.4 ± 4.9	$p=0.9$	$p=0.8$
Parafoveal DCP	60.8 ± 2.4	60.55 ± 1.7	$p=0.06$	61.47 ± 2.3	$p<0.003$	$p=0.5$

FT: foveal thickness, PFT: parafoveal thickness, SCP: superficial capillary plexus, DCP: deep capillary plexus.

After dividing the study population by sex, in the female group we found significant differences between prepubertal and pubertal children in foveal SCP (34.35 ± 5.4 vs 31.05 ± 5.5, $p<0.007$). Between pubertal and postpubertal girls we observed differences in DCP foveal (30.23 ± 6.1 vs 32.7 ± 4.7, $p<0.03$) and parafoveal (62.04 ± 2.2 vs 60.79 ± 1.6, $p<0.003$). Analysis of young males revealed significant differences in whole SCP between prepubertal and pubertal subjects (52.5 ± 2.3 vs 51.21 ± 2.9, $p<0.05$) and between prepubertal and post-pubertal subjects in whole SCP (52.5 ± 2.3 vs 50.32 ± 2.8, $p<0.001$) and parafoveal SCP (respectively 53.83 ± 2.6 vs 51.89 ± 3.1, $p<0.01$). We recorded no statistical differences between pubertal and postpubertal males.

To determine the influence of pubertal status on retinal parameters, without a possible influence of diabetes duration we matched the groups until they were statistically the same (Kruskal-Wallis $H=4.9$, $p>0.9$). In such matched groups, we observed the differences between prepubertal (eyes $n=70$), pubertal (eyes $n=95$), and postpubertal (eyes $n=70$) children in FT (pub to post, 254.1 ± 18.4 vs 261.11 ± 19.1, $p<0.02$, and pre to post, 249.13 ± 17.6 vs 261.11 ± 19.1, $p<0.0002$), PFT (pre to post, 316.2 ± 16.1 vs 321.4 ± 14.3, $p<0.05$), whole SCP (pre to post, 53.67 ± 2.1 vs 50.67 ± 2.7, $p<0.04$), foveal SCP (pre to

pub, 34.03 ± 5.2 vs 32.09 ± 5.2, $p<0.04$, and pre to post, 34.03 ± 5.2 vs 32.44 ± 4.4, $p<0.05$), parafoveal SCP (pre to post, 53.4 ± 2.98 vs 52.2 ± 3.0, $p<0.02$, and pub to post, 53.3 ± 3.3 vs 52.2 ± 3.0, $p<0.04$), and parafoveal DCP (pre to post, 61.7 ± 2.5 vs 60.7 ± 1.7, $p<0.02$, pub to post, 61.54 ± 2.2 vs 60.7 ± 1.7, $p<0.01$).

Discussion

In this study, we performed a detailed analysis of retinal vessel density in diabetic children using non-invasive OCTA. In the last 20–30 years, several studies have reported a declining incidence in DR in children with diabetes, from 49% in the early 1990s to 24% in the early 2000s. Harvey[13] found that post pubertal patients have a 3.2 fold greater risk of retinopathy than patients before puberty. Many authors revealed that puberty contributes to the development of late metabolic complications of diabetes.[14] In the multicenter study on 1032 Turkish children with T1D, DR was present in 1.95% of pubertal patients and 0.5% of prepubertal patients.[15] Furthermore, a recent large US study has shown an increase of 20% (95% CI 6%–35%) of the hazard of DR for every one-point rise of HbA1c in children with T1D, and what is very important is that DR in children seems to progress rapidly.[16] However,
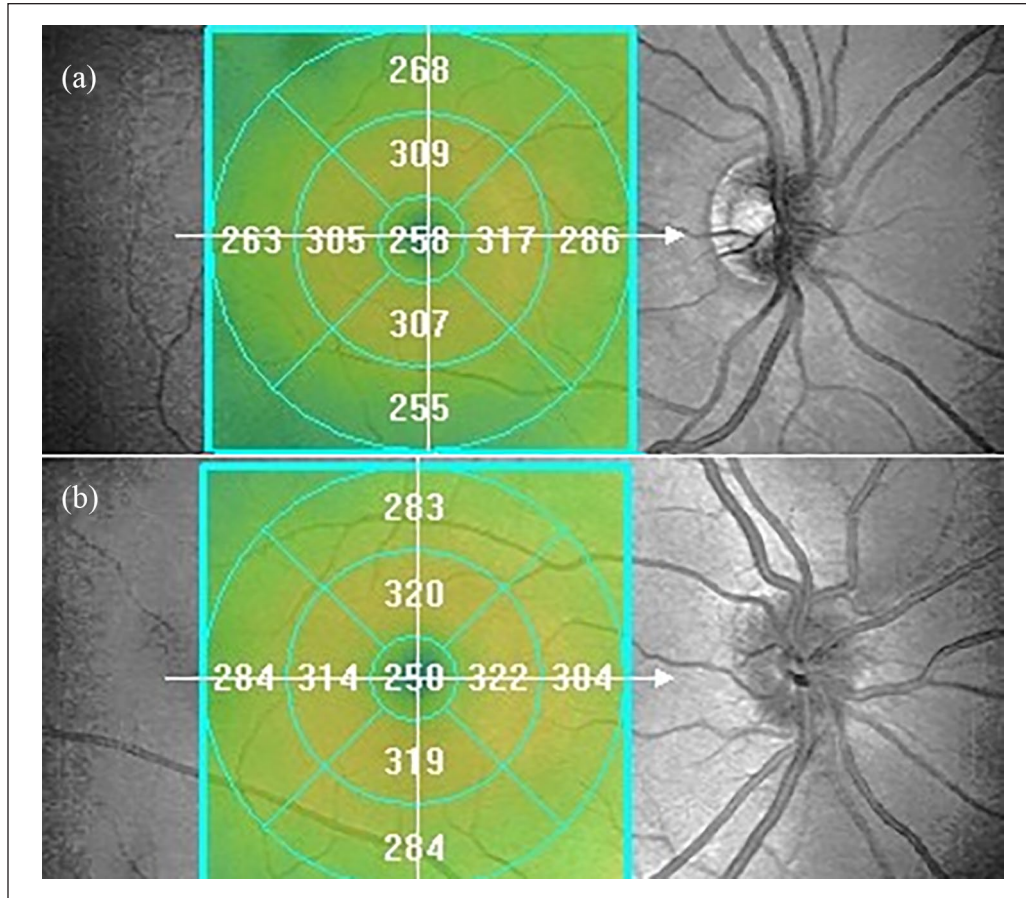
Figure 1. Representative macular thickness maps (FT and PFT) of pubertal (a) and prepubertal patients (b).
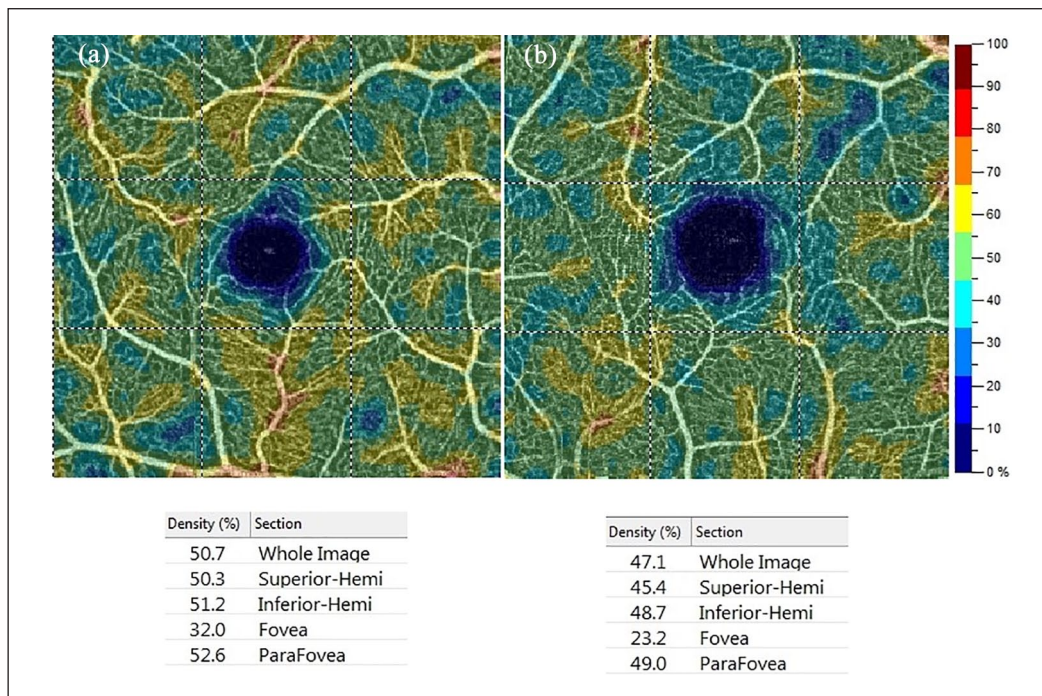

Figure 2. Representative OCTA images of vessel density in superficial capillary plexus in prepubertal (a) and postpubertal subjects (b).
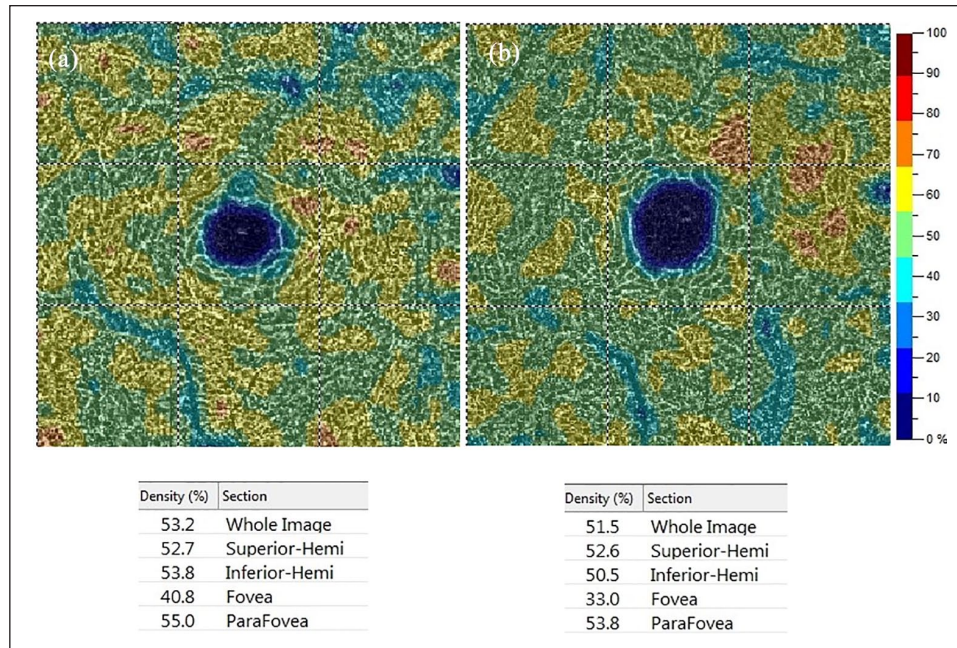
Figure 3. Representative OCTA images of vessel density in deep capillary plexus in pubertal (a) and postpubertal subjects (b).

it may be still controversial if all of the above is related to longer duration of T1D or to the influence of puberty. To eliminate any doubts we have decided to carry out an additional analysis to compare children with the same duration of diabetes. Its results confirmed our previous findings, that is, negative influence of puberty on OCTA results. In our study, the mean duration of diabetes was short, about 6 years in the postpubertal group or less in the pubertal and prepubertal subgroups and no child had any signs of DR on fundus examination and color fundus photography. Our participants were a little younger ($<$18) than Wang's ($<$21)[17] and Mameli's ($<$25),[10] who diagnosed a few cases with DR in their studied groups. In our group, we detected a significant increase of foveal thickness during puberty. These results are consistent with Demir et al.,[11] who also found retinal thickening in diabetic children in the pubertal stage. Read et al.[18] on the large population of healthy children revealed that foveal thickness increased significantly with age, with a mean increase of 1.8 µm per year in childhood. Therefore it is possible that the increase in retinal thickness may be related to age and puberty status and not to diabetes. A few studies focused on alterations in macular vessel densities in diabetic youths, but they did not analyse the influence of puberty. Demir et al.[11] revealed that the vessel density in SCP and DCP was lower in diabetic children, but the correlation was not statistically significant. Li et al.[19] proved a substantial reduction in macular vessel density in children with T1D. Similarly, Mameli et al.[10] found lower vessel density in SCP and DCP in diabetes children and adolescents. We did not observe any differences between vessel densities in superficial and deep

capillary plexuses in the whole group. Still, this correlation was found in foveal SCP between prepubertal and pubertal children, as well as between prepubertal and postpubertal ones. Even more differences were confirmed when we analyzed the groups of children matched depending on diabetes duration. In this group, parafoveal vessel density both in superficial and in deep retinal plexuses, as well whole SCP were decreased during puberty. Therefore, the statistically significant decrease of foveal vessel density from prepubertal through puberty to a postpubertal phase of growth must be highlighted. According to earlier studies, pubertal girls have a higher risk of developing diabetic retinopathy than boys, but we confirmed the early changes in retinal microcirculation both in girls and boys.[3] The main strength of the study is a large sample of patients—to the best of our knowledge, the current study described the largest group of diabetes children compared to the previous reports. However our study was subject to some limitations. Since this was a single-center study, all the subjects were Caucasian, and there were no differences in the clinical population, our results may not necessarily reflect or refer to the entire world cohort of T1D children.

In conclusion, our data suggest that in a cohort of pubertal children with short duration of T1D, alterations in retinal vessel density occur early, and progress during puberty before the onset of other diabetes-related complications. It is therefore possible that these changes may be an early indicator for the development of retinopathy. If further studies confirm these findings, OCTA may perhaps be considered a part of screening in children with T1D for early detection of microvascular abnormalities. Longitudinal

observation of these children is necessary to identify their retinal microcirculation in the future and determine if any of these OCTA measurements are predictive of the severity of DR.

Author contributions

Conception or design: M.W.-M., J.G. Acquisition, analysis, or interpretation of data: M.W.-M., J.G., M.B.-W., A.O., A.B. Drafting the work or revising: M.W.-M., J.G., M.B.-W., M.S. Final approval of the manuscript: M.W.-M., J.G., M.B.-W., A.O., A.B., M.S.

Declaration of conflicting interests

Funding

ORCID iDs

Marta Baszyńska-Wilk https://orcid.org/0000-0001-8741-3518
Andrzej Olechowski https://orcid.org/0000-0003-2089-4518

References

1. Holl RW, Lang GE, Grabert M, et al. Diabetic retinopathy in pediatric patients with type-1 diabetes: effect of diabetes duration, prepubertal and pubertal onset of diabetes, and metabolic control. *J Pediatrics* 1998; 132(5): 790–794.

2. Donaghue KC, Fairchild JM, Craig ME, et al. Do all prepubertal years of diabetes duration contribute equally to diabetes complications? *Diabetes Care* 2003; 26: 1224–1229.

3. Cho YH, Craig ME and Donaghue KC. Puberty as an accelerator for diabetes complications. *Pediatr Diabetes* 2014; 15: 18–26.

4. Thomas RL, Dunstan FD, Luzio SD, et al. Prevalence of diabetic retinopathy within a national diabetic retinopathy screening service. *Br J Ophthalmol* 2015; 99: 64–68.

5. Porta M and Allione A. Diabetic retinopathy and its relevance to paediatric age. An update. *Pediatr Endocrinol Rev PER* 2004; 1: 404–411.

6. Olsen BS, Sjølie AK, Hougaard P, et al. The significance of the prepubertal diabetes duration for the development of retinopathy and nephropathy in patients with type 1 diabetes. *J Diabetes Complications* 2004; 18: 160–164.

7. Jia Y, Tan O, Tokayer J, et al. Split-spectrum amplitude-decorrelation angiography with optical coherence tomography. *Opt Express* 2012; 20: 4710–4725.

8. Gołębiewska J, Olechowski A, Wysocka-Mincewicz M, et al. Optical coherence tomography angiography vessel density in children with type 1 diabetes. *PLoS One* 2017; 12: e0186479.

9. Niestrata-Ortiz M, Fichna P, Stankiewicz W, et al. Enlargement of the foveal avascular zone detected by optical coherence tomography angiography in diabetic children without diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2019; 257: 689–697.

10. Mameli C, Invernizzi A, Bolchini A, et al. Analysis of retinal perfusion in children, adolescents, and young adults with type 1 diabetes using optical coherence tomography angiography. *J Diabetes Res* 2019; 2019: 5410672.

11. Demir ST, Ucar A, Elitok GK, et al. Evaluation of retinal neurovascular structures by optical coherence tomography and optical coherence tomography angiography in children and adolescents with type 1 diabetes mellitus without clinical sign of diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2020; 258: 2363–2372.

12. Tanner JM and Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 1976; 51: 170–179.

13. Harvey JN. The influence of sex and puberty on the progression of diabetic nephropathy and retinopathy. *Diabetologia* 2011; 54: 1943–1945.

14. Hietala K, Harjutsalo V, Forsblom C, et al. Age at onset and the risk of proliferative retinopathy in type 1 diabetes. *Diabetes Care* 2010; 33: 1315–1319.

15. Şimşek DG, Aycan Z, Özen S, et al. Diabetes care, glycemic control, complications, and concomitant autoimmune diseases in children with type 1 diabetes in Turkey: a multicenter study. *J Clin Res Pediatr Endocrinol* 2013; 5: 20–26.

16. Hamid A, Wharton HM, Mills A, et al. Diagnosis of retinopathy in children younger than 12 years of age: implications for the diabetic eye screening guidelines in the UK. *Eye (Lond)* 2016; 30: 949–951.

17. Wang SY, Andrews CA, Herman WH, et al. Incidence and risk factors for developing diabetic retinopathy among youths with type 1 or type 2 diabetes throughout the United States. *Ophthalmology* 2017; 124: 424–430.

18. Read SA, Collins MJ, Vincent SJ, et al. Macular retinal layer thickness in childhood. *Retina (Philadelphia, Pa)* 2015; 35: 1223–1233.

19. Li T, Jia Y, Wang S, et al. Retinal microvascular abnormalities in children with type 1 diabetes mellitus without visual impairment or diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2019; 60: 990–998.