PRE-RETINOPATHY OF TYPE 1 DIABETES WITHIN THE CONTEXT OF FUNCTIONAL, STRUCTURAL AND MICROCIRCULATORY CHANGES IN THE MACULAR REGION

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Dedicated to the memory of professor Jan Vavřinec, MD, PhD (1947–2016), co-founder of Czechoslovak paediatric diabetology, on the fifth anniversary of his death.

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SUMMARY

Aim: The authors assessed the development of intraocular changes in type 1 diabetes (T1DM) from the onset of the disease leading to diabetic retinopathy (DR). The quote: "There must be an intermediate stage between the physiological intraocular finding and the diabetic retinopathy itself ", (prof. Jan Vavřinec).

Methods: A two-year study (2018 and 2019) was conducted at the Department of Ophthalmology of the Teaching Hospital Kralovske Vinohrady in Prague (Czech Republic). There were 54 patients aged 17–42 years, the detection of T1DM ranged between the 1st and 14th year of life, with a duration of 12–35 years. Individual patients were always examined simultaneously by three methods: CS (contrast sensitivity), SD-OCT (spectral domain optical coherence tomography) and OCT-A (optical coherence tomography-angiography). We examined 106 eyes once and in a comprehensive manner.

Results: We have shown that there is an intermediate stage between the physiological finding on the retina and DR, so-called diabetic pre-retinopathy (DpR). Subsequent redistribution of the observed into two DpR subgroups was derived from the size of the FAZ, either with its smaller area or with a larger area determining the microvascularity of the central area of the retina. The results of both other methods were assigned to these values. For SD-OCT, the depth of the fovea (the difference between the central retinal thickness and the total average retinal thickness) was determined, which was affected by the increased the macular cubature. In all patients it was on average 10.3 μm^3. The retina in the central area was significantly strengthened compared to the healthy population at the level of significance $p \leq 0{,}001$. We divided the actual DpR into an image: DpR1 in 26.5 % of eyes – condition with an average shallower fovea only by 21.5 µm below the level of the surrounding retina and an average narrower FAZ: 0.165 mm^2 and with a more significant decrease in CS; DpR2 in 40.5 % of eyes – condition with average deeper fovea by 42 µm, i.e., more significantly and average larger FAZ: 0.325 mm^2 with lower decrease of CS. At the same time, other changes in microvascularity were noted, such as disorders in the sense of non-perfusion in the central part of the retina of various degrees. This finding differed significantly from changes in already established (non-proliferative) NPDR in 36 % of eyes, when a significant decrease in CS with normal visual acuity was found 4/4 ETDRS. Statistical differences in CS between DpR1 and DpR2 and NPDR were determined – always $p \leq 0.001$. The average depth of the fovea was NPDR: 29.5 µm. NPDR had the largest average FAZ: 0.56 mm^2. Also significant were the most significant changes in non-perfusion and especially the presence of microaneurysms.

Conclusions: These three non - invasive methods helped to monitor the dynamics of the development of ocular changes in T1DM of better quality than the determination of visual acuity and ophthalmoscopic examination. Increased retinal volume induced hypoxia of visual cells with subsequent dual autoregulatory mechanism conditioning two types of diabetic pre-retinopathy before the onset of DR.

Key words: contrast sensitivity, diabetic retinopathy, OCT-A, SD-OCT, T1DM

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INTRODUCTION

Within the framework of the development of ocular changes conditioned by T1DM (type 1 diabetes mellitus), the physiological finding on the retina passes into the stage of pathological diabetic retinopathy (DR). The clarification of the pathogenesis and pathophysiology of DR is connected with Cunha-Vaz's discovery using fluorophotometry at the end of the 1970s [1,2]. The method enabled a description of the collapse of the blood-ocular barrier and led to the introduction of the term pre-retinopathy [3], which refers precisely to this intermediate stage. DR itself is defined by its symptoms, which include microaneurysms (MA), haemorrhages, phlebopathy, intraretinal abnormalities and cotton wool spots. DR is divided into several degrees. The most severe affliction with regard to visual acuity is represented by the presence of diabetic macular edema (DME) [3,4]. Diabetic pre-retinopathy (DpR) is linked primarily with changes of the macular region due to the metabolic demand for nutrition of this region. It has already been described as microangiopathic abnormalities with irregularities of the capillaries, their tortuosity and dilation [5,6,7,8], extending deeper into the avascular foveola. Other symptoms of DpR include change of the pattern of the macular region, "flecked" retina, which is represented by a loss of the foveal reflex and change of pigmentation with lighter areas resembling a map, as illustrated in fig. 1. Symptoms may also include the presence of punctiform hard exudates (HE) [6,7], which are macrophages full of lipoproteins [3].

In the last two decades we have engaged in the research of DpR in T1DM with regard to complex changes in contrast sensitivity (CS) [7,9]. Further pilot studies have compared changes of a physiological retina with DpR and NPDR (non-proliferative diabetic retinopathy) with the aid of SD-OCT (spectral-domain optical coherence tomography) [10] and OCT-A (optical coherence tomography-angiography) [11]. The content of this study is an assessment of DpR with regard to complex mutual functional changes with the aid of CS, structural changes with the use of SD-OCT, and microcirculatory changes upon an assessment of findings using OCT-A.

COHORT AND METHOD

In the years 2018–2019, 54 patients with T1DM were examined at the Department of Ophthalmology at the Královské Vinohrady University Hospital in Prague. The cohort comprised 28 young men and 26 young women within the age range of 17–42 years, average age 24.6 years. The onset of the metabolic disorder began within the age range of one to fourteen years of age, average 5.5 years, and the period of its duration was 12–35 years, average 18.4 years. A comprehensive examination incorporated determination of optimal visual acuity (VA), which was 4/4 ETDRS naturally or with optimal correction (in isolated cases with an error of 1–2 letters), assessment of transparency of the lens on a slit lamp, biomicroscopic examination of the ocular fundus, as well as direct ophthalmoscopy in order to observe details in the macular region. The evaluated examination at the given age had to include simultaneously performed CD, SD-OCT and OCT-A.

CS is a functional examination which better detects the condition of the visual analyser than examination of central VA. It provides information about the quality of processing of the given optical stimulus, and about different spatial frequencies sent from the photoreceptor cells of the retina to the visual centre in the cerebral cortex via various channels. The magnocellular system dominates in the processing of low-frequency threshold stimuli of fundamental and chromatic CS, whereas the parvocellular system primarily transmits data of high-frequency stimuli with a high spatial resolution [12]. We evaluated CS with the aid of the instrument CSV 1000 (Vector Vision, USA) with four double rows (pairs) of circular stimulus targets with spatial frequencies of 3, 6, 12 and 18 cycles per angle degree (cpd). In addition to the pattern, there is always a total of 8 columns of targets, with a decreasing level of contrast. We performed the examination in a room with constant lighting of 450 lux, and the instrument was calibrated to a photopic level of 85 cd/m^2. The last correctly determined target was evaluated as the threshold contrast. Targets of 18 cpd represent the foveola, 12 cpd the foveal region, 6 cpd the parafoveal region and 3 cpd the perifoveal region [13]. The diameter of the foveola is 350 µm, the diameter of the central fovea is 1500 µm, of the parafoveal region 2500 µm and of the perifoveal region 5500 µm [14]. As the designated standard we set the following: for 3 cpd 6.4 ±0.4 (A), with the borderline value of the 6th target; for 6 cpd 7.1 ±0.3 (B), for 12 cpd 7.1 ±0.5 (C) and finally for 18 cpd 7.2 ±0.6 (D). For all these three cycles the borderline value is represented by the 7th target. With regard to the fact that this concerns an exponential function within the framework of an evaluation of the individual cycles and not a linear curve, it has the form of an incomplete exponential spiral. The measured values for a mutual evaluation are converted into a logarithmic relationship by means of a shift of 0.15 log per one target and one cycle according to the recommendations of the manufacturer [13]. The actual standard for the age of 11–19 years became the basis for evaluation of CS in our study, and we used it also for young diabetics. The range for adults stated by the manufacturer is for all age groups in adulthood. Due to its dispersion it is unsuitable for younger individuals. Another outcome was the norms for the age group of 6 to 11 years, and both became the content of the grant IGA NR/7952-3 [15]. Furthermore, both CS standards were incorporated in the source materials within the framework of the manufacturer's recommendations [13].

SD-OCT is an analogy of the B-scan in ultrasound examination. The principle is low-coherence interferometry, which measures the distance of various structures inside the tissue with high sensitivity to the light signal reflected from the structures of the eye. It represents a method of three-dimensional evaluation of the structures of the retina and choroid [15]. For the examination we used

the instrument Cirrus OCT (Carl Zeiss, Germany). We included the cubic content of the measured retinal cube in the delineated quadrant of the macular region of 36 mm^2 within the measured SD-OCT parameters. This evaluates any applicable thickening of the central part of the retina of a larger extent better than separate central retinal thickness alone. The second statistically evaluated parameter was the actual depth of the fovea itself, as the difference between central retinal thickness and average retinal thickness. The relationship evaluated the character of the shape of the foveola in relation to any applicable retinal thickening, which influence oxygenation of the retina in the given area better than the above-stated two retinal thicknesses, from which it is not possible to deduct this influence. We also evaluated the presence of HE and present DME. We calculated the norm for the content of the cube of the central part of the retina of 6 x 6 mm2 at 10.0 ±0.3 µm3. We measured central retinal thickness as follows: 240 ±17 µm, and average retinal thickness 281 ±10 µm. The calculation of the resulting depth of the foveola was determined by the deduction of central from average retinal thickness, and represented -32 ± 15 µm, since in physiological condition the foveola is beneath the level of the remainder of the retina due to its depression [10]. The minus sign explains this condition, in contrast with DME, which is above the level of the central part of the retina including the fovea, which indicates a plus value.

OCT-A evaluates the quantitative and qualitative haemodynamics of the vessels of the retina and choroid, by means of quantitative measurement of the flow speed of blood attained by the use of the Doppler technique, working on the principle of variable reflectivity on membranes of flowing blood elements [17]. To display the retinal microvasculature by OCT-A examination we used the instrument Spectralis (Heidelberg Engineering, Germany/USA). For measurement we used our own specific setting, in which the instrument recorded 7 images in the same cross-section of the retina in high-resolution mode. By this method we displayed a surface of 15 x 15 degrees, which corresponds to an approximately 4.4 x 4.4 mm cross-section of the retina and choroid. In the given setting we arrived at a compromise between the necessary quality of imaging of the vessels and the hardware capacities of the instrument. Segmentation of the retinal layers took place automatically, and in all cases correctly (manual correction was not required). In order to attain the most flawless and most contrasting imaging of the foveal avascular zone (FAZ) we selected a combined image from all the vascular complexes of the retina, namely the superficial vascular complex (SVC), the intermediate vascular complex (IVC) and finally the deep vascular complex (DVC). By using the inbuilt function "Draw Region" it was possible to manually delineate the region of the FAZ, and using this method we calculated its surface area. For the norm of the healthy young population we set a surface area of the FAZ with the size of 0.253 ±0.092 mm^2. In general, the pathological value of the FAZ in T1DM without specification of the ocular finding was 0.300 +0.132 mm^2. We also evaluated the shape of the FAZ, capillary activity in the surrounding area and the presence of any potential MA.

DpR was manifested by a fairly regular shape of the FAZ, whereas in NDPR the shape was irregular. The difference in capillary activity and its character and surface area divided DpR into two types: DpR1 (narrower FAZ) and DpR2 (wider FAZ). DpR1 was accompanied by irregularity of the parafoveal capillaries with signs of non-perfusion in the SVC, whereas in DpR2 the zones of non-perfusion were more numerous, and furthermore here there was a sign of an overlap of capillary bundles into the FAZ, which was absent in the case of DpR1. The finding in the DVC differed in the degree of thinning of the capillary network, since in DpR2 it was more pronounced than in DpR1. In NPDR the FAZ was irregular, with multiple zones of non-perfusion with MAs in the SVC and with dilation of the capillary network in the DVC [11].

Fig. 2 shows a physiological finding on the ocular fundus in a healthy 26-year-old patient with Hb1Ac 38 mmol/mol and a range of CS standard with a graph of cycles within the physiological range, SD-OCT with depth of the foveola of -37 µm and FAZ (OCT-A) with a surface area of 260 mm^2 in an image of combined vascular segments.

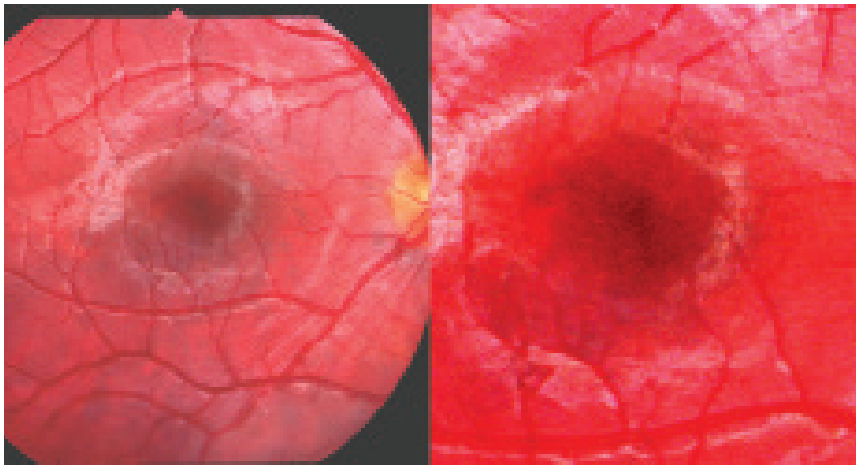


Figure 1. Left: image of map-like pigmentation and non-pigmentation in central area of retina and right: detail of "flecked macula"

RESULTS

Tables 1 and 2 summarise the time factors of T1DM in relation to DpR and NPDR with the absolute values of CS, SD-OCT and OCT-A in the individual patients in this 2-year study. DpR is divided in the table into the upper section for type 1 DpR and the lower section for type 2 DpR, in order to clearly present the differences in the individual parameters. We observed this metabolic disorder with potential

Table 1. Time factors of T1DM and diabetic pre-retinopathy absolute values: CS cycles per degree (cpd) and SD-OCT: macular volume (μm^3) and foveolar depth (μm) and OCT-A (FAZ – mm^2)

order & sex	time factors (years)			CS: cicles of row		SD-OCT		OCT-A	
	origin T1DM	origin DpR	duration T1DM	3.6.12.18 (cpd)		volume & depth		FAZ	
				OP	OL	OP	OL	OP	OL
1 & M	6	18	15	5.6.7.7	6.7.7.7	10.2 & -5	10.0 & -3	0.06	0.05
2 & M	7	17	13	6.7.7.7	6.7.7.7	10.6 & -18	10.6 & -29	0.16	0.15
3 & M	7	18	15	6.6.4.5	not possible	10.6 & -6	11.1 & -14	0.21	0.19
4 &. F	5	19	15	6.7.7.7	6.7.7.7	11.9 & -31	11.6 & -29	0.16	0.15
5 & M	14	23	23	6.5.7.7	6.6.7.7	11.1 & -35	11.3 & -30	0.12	0.12
6 . M	3	15	13	7.7.7.6	7.7.7.7	9.3 & -18	9.5 & -19	0.22	0.23
7 & F	9	19	12	6.6.6.5	6.6.5.5	10.4 & -21	10.4 & -25	0.17	0.16
8 & F	2	13	17	5.4.4.5	5.4.4.4	10.2 & -42	10.1 & -42	0.22	0.25
9 & M	5	15	14	6.6.5.6	6.7.6.6	10.4 & -13	10.4 a -21	0.12	0.11
10 & M	1	14	16	6.7.7.7	6.7.7.8	11.0 & -26	10.9 & -24	0.23	0.2
11 & F	7	17	13	5.5.5.6	5.6.6.6	9.8 & -10	9.6 & -9	0.12	0.09
12 & M	4	17	14	6.7.5.6	6.7.6.7	10.0 & -35	10.1 & -39	0.2	0.23
13 & M	3	15	14	6.7.7.7	6.7.7.8	10.7 & -22	10.9 & -23	0.27	0.24
14 & M	7	19	12	6.6.6.5	5.6.6.6	9.4 & -11	9.3 & -9	0.15	0.17
Type 1 DpR according to OCT-A (top)					Type 2 DpR according to OCT-A (below)				
15 & F	4	16	21	6.7.7.7	6.7.7.6	10.7 & -60	10.7 & -60	0.41	0.36
16 & F	4	15	15	6.7.6.6	6.5.5.5	9.9 & -40	10.2 & -41	0.39	0.31
17 & F	6	14	13	not possible	6.6.8.8	10.8 & -59	10.6 & -54	0.39	0.42
18 & M	3	19	20	5.7.6.5	5.7.6.6	10.0 & -32	10.1 & -32	0.22	0.24
19 & F	6	17	20	6.6.6.6	6.6.5.6	10.1 & -50	10.2 & -43	0.41	0.38
20 & M	8	18	20	6.6.7.7	5.7.6.6	10.1 & -26	10.1 & -28	0.43	0.37
21 & M	5	18	25	6.7.6.7	6.7.6.6	9.0 & -48	9.0 & -44	0.54	0.5
22 & M	3	17	20	6.6.7.6	6.7.7.7	10.5 & -19	11.0 & -37	0.22	0.23
23 & M	3	16	15	6.7.7.7	6.7.7.7	9.7 & -24	9.7 & -26	0.25	0.26
24 & F	4	19	17	6.6.6.6	6.6.5.6	10.1 & -41	10.0 & -34	0.31	0.32
25 & M	6	16	13	6.7.7.8	6.6.7.8	9.8 & -21	9.8 & -20	0.28	0.31
26 & F	5	16	13	6.5.5.5	6.5.7.5	9.8 & -52	9.6 & -67	0.47	0.46
27 & M	6	17	13	6.7.6.5	6.6.6.5	10.5 & -39	10.4 & -41	0.24	0.26
28 & M	3	17	22	7.7.7.8	7.7.7.8	10.9 & -33	10.9 & -41	0.36	0.42
29 & M	9	20	14	6.7.7.6	6.7.6.6	10.4 & -44	10.3 & -49	0.23	0.26
30 & F	2	15	21	6.5.5.5	6.5.5.6	11.0 & -43	11.1 & -37	0.29	0.31
31 & M	8	22	29	7.8.7.7	6.7.7.6	10.5 & -59	10.5 & -62	0.34	0.33
32 & M	12	23	16	6.7.8.7	6.7.7.7	10.9 & -46	10.6 & -42	0.44	0.4
33 & F	9	21	21	6.7.7.6	6.7.7.7	10.7 & -42	10.7 & -46	0.32	0.35
34 &.M	3	17	15	7.7.7.7	6.6.7.7	11.0 & -38	10.9 & -39	0.25	0.28
35 & F	4	16	14	6.6.6.5	6.8.8.7	10.2 & -40	10.3 & -49	0.29	0.29
36 & F	4	17	16	5.5.7.6	5.6.6.6	9.7 & -25	9.6 & -23	0.37	0.36

ocular symptoms for 12–17 years, on average 15 years. We diagnosed the first symptoms of the onset of DpR with the aid of direct ophthalmoscopy within a period of duration of T1DM of 14–23 years, on average 17.4 years, and they were followed by intraocular manifestations of NPDR appearing within a period of duration of the disease from 17 to 31 years, on average 23 years.

CS: Examination on all the patients demonstrated changes in the quality of the values (Table 3). In a comparison of the individual cycles per degree, the value for DpR1, DpR2 and NPDR of 3 cpd was borderline. For DpR of both types the value of 6 cpd was also borderline, but the remaining cycles (12 and 18 cpd) were reduced. In NPDR the cycles of 6, 12 and 18 cpd were always reduced. The mutual statistical difference between the individual types of ocular affliction was significant: $p \leq 0.001$. The smallest difference of $p \leq 0.001$ was in the relations of DpR1 versus (vs) DpR2, and the largest difference of $p \leq 0.0001$ was in the relation of DpR1 vs NPDR.

SD-OCT: Cubature for DpR1 was within the range of 9.3–11.9 μm^3, on average 10.4 μm^3, and for DpR2 this was 9.0–1.1 μm^3, on average 10.3 μm^3. In the case of NPDR the value did not differ markedly, specifically from 9.3 to 11.2 μm^3, on average 10.2 μm^3 (Graph 1). A statistical comparison did not show any fundamental difference: DpR1 vs DpR2 – $p = 0.198$, DpR1 vs NPDR – $p = 0.461$ and DpR2 vs NPDR – $p = 0.132$. From this it ensues that thickening (through infiltration) of the central part of the retina throughout the period of duration of T1DM from 14 to 35 years was analogous, with an average of 10.3 μm^3. This was a fundamental difference in comparison with a physiological retina in subjects observed without a metabolic disorder (10.0 ±0.3 μm^3), on a level of significance of $p \leq 0.001$. The depth of the foveolas manifested fundamental changes, in the case of DpR1 within the range of -42 µm to -3 µm, on average -21.5 µm, whereas in DpR2 the dispersion was greater, from -67 µm to -19 µm, with an average of -41 µm. The largest dispersion was manifested in NPDR from -74 µm to +35 µm, on average -29.5 µm (Graph 2). The stated values also showed a pronounced statistical difference: DpR1 vs DpR2 – $p = 0.007$, DpR1vs NPDR – $p \leq 0.001$and DpR2 vs NPDR – $p = 0.034$. These differences were conditioned by the representation of shallow foveolas beneath -17 µm in 36 % of eyes in DpR1 (no deeper foveola was identified in this type of DpR) and only confirmed deeper foveolas above -47 µm

Table 3. Logarithmic levels of cycles per degree (cpd) in row of CS

CS	Optimal logarithmic levels of circular stimulus targets		
Spatial frequencies	DpR1	DpR2	NDPR
3 cpd	1.76	1.75	1.74
6 cpd	2.05	2.06	1.9
12 cpd	1.73	1.62	1.58
18 cpd	1.31	1.43	1.16
diametr	1.71	1.72	1.6
deviation	0.26	0.23	0.28

Table 2. Time factors of T1DM and NPDR with absolute values: CS cycles per degree (cpd) and SD-OCT: macular volume (μm^3) and foveolar depth (µm) and OCT-A (FAZ – mm^2)

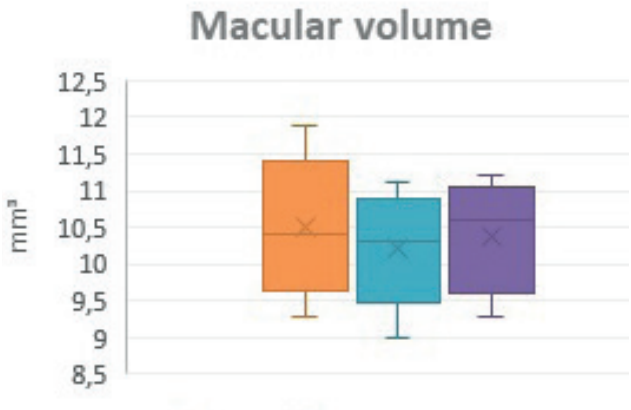
order & sex	time factors (years)			CS: cickes of row		SD-OCT		OCT-A	
	origin T1DM	origin NDPR	duration T1DM	3,6,12,18 (c./st.)		volume & depth		FAZ	
				OP	OL	OP	OL	OP	OL
1 & F	5	21	25	6.6.6.7	6.6.6.7	9.5 & -25	9.9 & -45	0.36	0.34
2 & M	11	22	21	5.6.5.5	6.6.6.7	11.2 & -16	11.2 & -24	0.15	0.18
3 & M	13	25	23	6.6.5.5	6.6.5.5	10.6 & -19	10.5 & -22	0.22	0.21
4 & M	3	17	18	6.6.6.6	6.6.6.7	10.6 & -74	10.8 & -67	0.46	0.48
5 & F	5	23	21	6.7.6.6	6.6.6.6	9.3 & -27	9.4 & -25	0.41	0.41
6 & F	5	31	30	5.5.3.3	5.5.4.4	10.8 & -32	11.0 &-34	0.2	0.29
7 & F	7	25	35	6.6.4.4	6.5.5.5	11.1 & -16	10.2 & -39	0.48	0.56
8 & F	3	23	33	6.5.5.6	6.5.4.6	9.7 & -18	9.8 & -34	0.21	0.35
9 & M	2	18	19	5.4.3.3	5.5.4.4	10.6 & -17	10.2 & -7	0.26	0.25
10 & F	5	24	20	6.6.7.8	6.6.7.6	10.9 & -65	10.9 & -67	0.49	0.44
11 & F	4	20	35	6.6.6.5	6.6.6.5	10.1 & -42	10.1 & -38	0.4	0.36
12 & F	11	27	31	6.7.5.5	6.7.6.6	10.2 & -19	10.0 & -19	0.41	0.42
13 & M	5	22	26	6.6.7.5	6.6.5.5	11.0 & -27	11.2 & -12	0.19	0.25
14 & M	3	26	29	6.5.4.5	6.6.6.5	10.9 & -36	11.0 & -36	0.23	0.24
15 & M	3	24	21	6.6.6.4	6.5.5.5	9.9 & -42	9.8 & -39	0.48	0.47
16 & M	10	24	17	5.5.5.4	5.6.5.5	9.9 & +27	9.8 & +35	0.06	0.06
17 & M	1	25	30	5.5.5.4	5.6.5.4	10.8 a -32	10.7 & -25	0.2	0.17
18 & F	4	18	15	6.7.6.7	6.7.7.8	10.9 & -63	10.7 & -66	0.38	0.41

in 27 % of eyes in DpR2. NPDR manifested both forms of foveolas, i.e. shallow and deeper, in the same percentage, specifically in 17 % of eyes.

OCT-A: The FAZ in its dimensions was similar to DpR2 and NDPR. It differed in the presence of MA, dilation of the capillaries together with a higher representation of avascular zones and irregularity of the shape of the FAZ only in the case of NPDR. The FAZ in DpR2 manifested a range of 0.06–0.54 mm^2, with an average of 0.325 mm^2, and in the case of NPDR the range was broader, from 0.06–0.565 mm^2, with an average of 0.56 mm^2. In DpR1 the range was narrower, from 0.05 to 0.27 mm^2, with an average of 0.165 mm^2 (Graph 3). As a result, the statistical evaluation was entirely insignificant between DpR2 and NPDR – $p = 0.212$, whereas it was fundamental between DpR1 vs DpR2 or DpR1 vs NPDR – $p \leq 0.001$.

Direct ophthalmoscopy versus OCT: The tortuosity of the end capillaries in the macular region was manifested on OCT-A by a constriction of the FAZ primarily in DpR1, but in isolated cases also in DpR2 or in NPDR. HEs were visible on the retina. In connection with "retinal flecking", hyperreflexive points appeared on the SD-OCT image, in some cases with an acoustic shadow in the outer plexiform layer in DpR and NPDR. MAs were perceptible on ophthalmoscopy as red, sharply bordered points on the retina in NPDR, better in red-free light. In the OCT-A image it was easier to detect MAs also due to the increase in their number in comparison with ophthalmoscopy.

SD-OCT versus OCT-A in context with CS: A comparison of two objective examination methods demonstrated a fundamental shallowing of foveolas of ≤ 17 µm on SD-OCT in 10 eyes with DpR1 (patients nos. 1, 2, 6, 9, 11, 14 – Table 2) and in 6 eyes with NPDR (patients nos. 2, 7, 9, 16 – Table 1). The value of shallowing was based on the value of the average norm upon deduction of the standard deviation. We did not detect any manifestation of fundamental shallowing of the foveolas in DpR2. By contrast, a pronounced deepening



Graph 1. Macular volume in diabetic pre-retinopathy 1,2 and NDPR

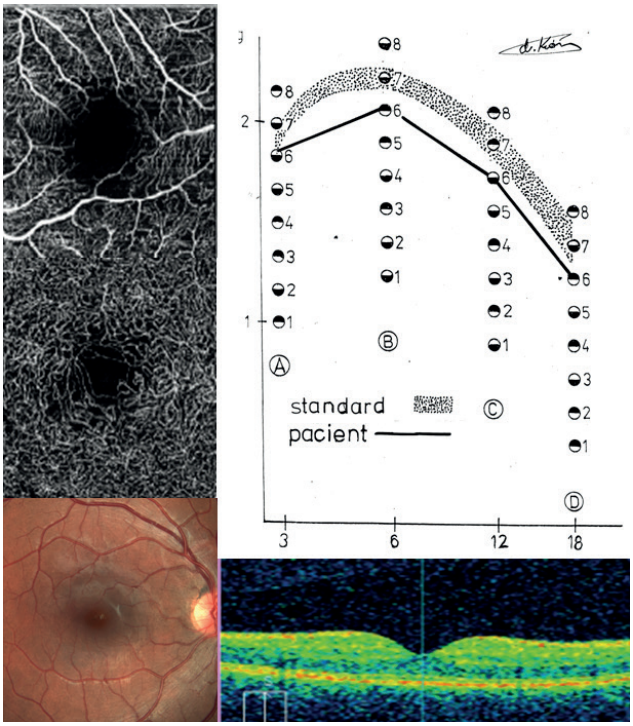
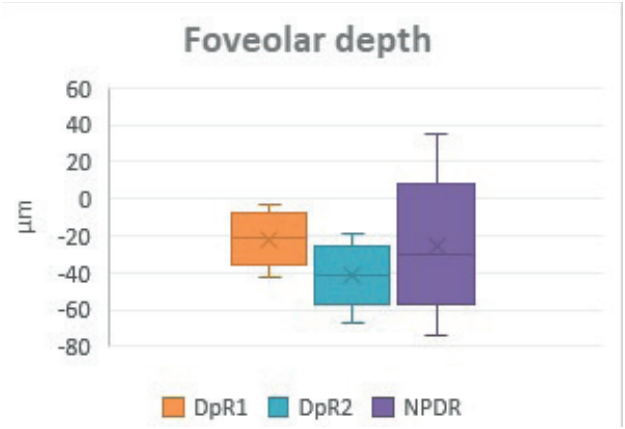


Figure 2. Physiological findings of three non-invasive examination methods: CS, SD-OCT, OCT-A (superficial/deep vascular complex – SVC/DVC) and photography of fundus



Graph 2. Foveolar depth in diabetic pre-retinopathy 1,2 and NDPR

Graph 3. FAZ in diabetic pre-retinopathy 1,2 and NDPR

of foveolas of ≥ 47 µm on SD-OCT was recorded in 10 eyes with DpR2 (patients nos. 15, 17, 19, 21, 29, 31 – Table 2) and in 6 eyes with NPDR (patients nos. 4, 10, 18 – Table 1). The value of deepening of foveolas was based on the average standard, by contrast with addition of the standard deviation. We did not demonstrate a deeper fovea in patients with DpR1. Assessment of the assigned subjective examination of CS was variable. In shallower foveolas and smaller surface areas of the FAZ, the examination of CS was accompanied by a decrease without a borderline value in all cycles

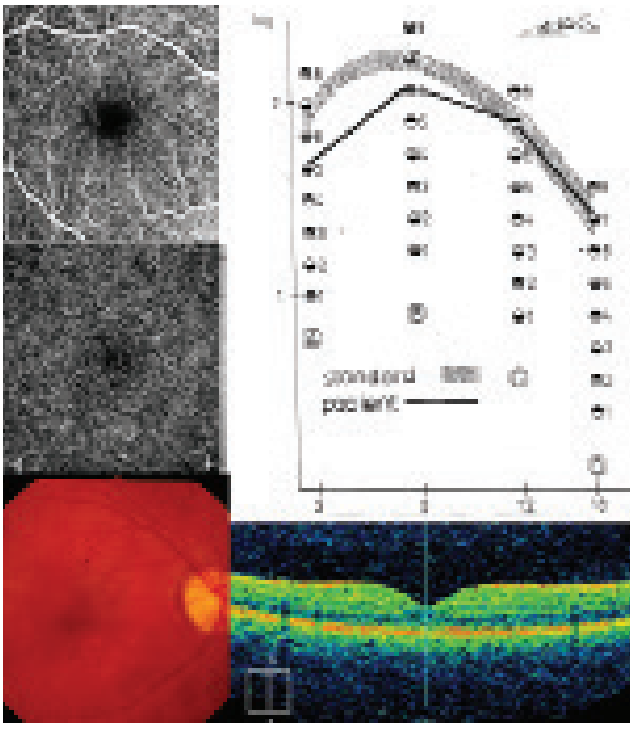


Figure 3. DpR1: CS, SD-OCT, OCT-A and fundus on patient 1 (Table 1)

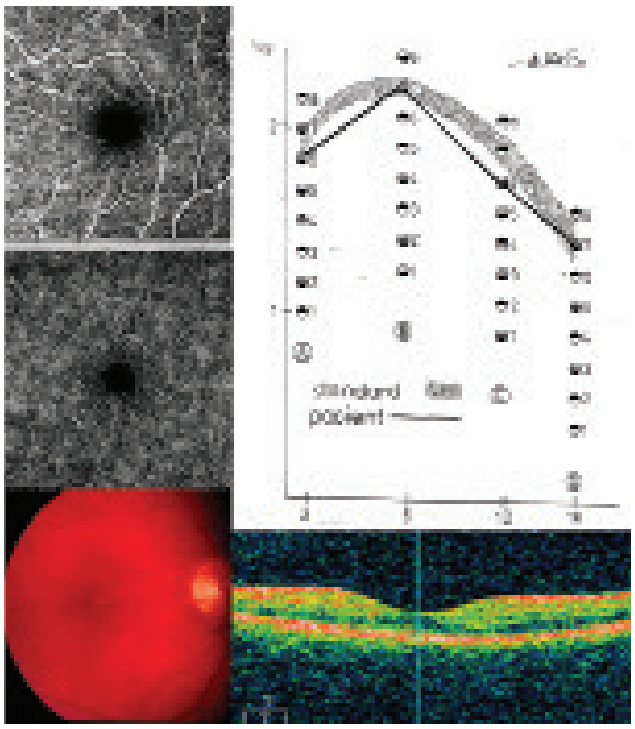


Figure 4. DpR2: CS, SD-OCT, OCT-A and fundus on patient 20 (Table 1)

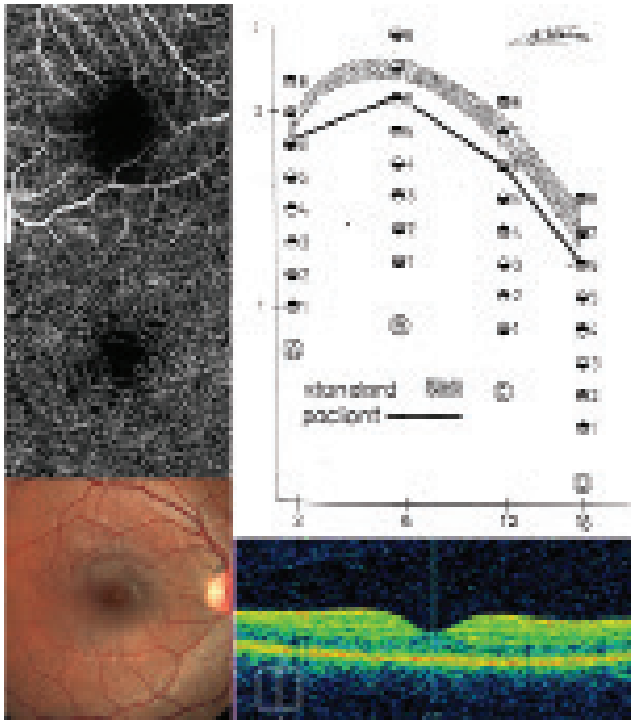


Figure 5. NPDR: CS, SD-OCT, OCT-A and fundus on patient 4 (Table 2)

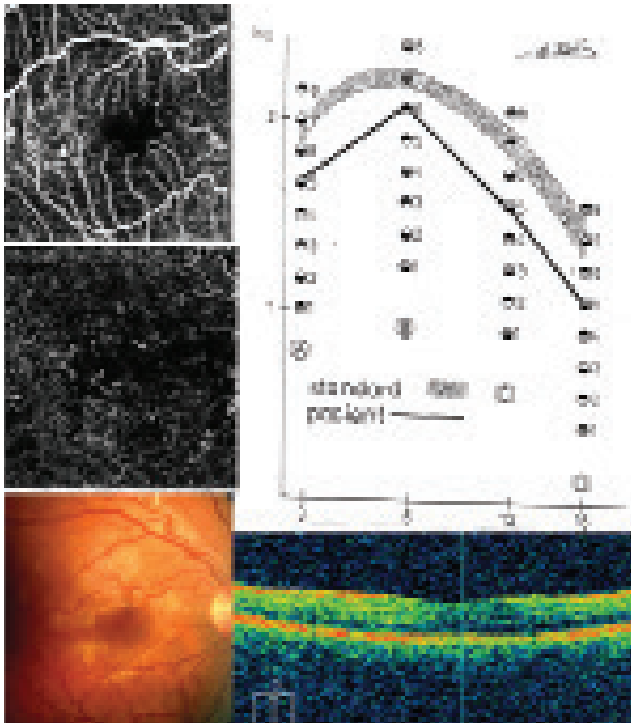


Figure 6. NPDR: CS, SD-OCT, OCT-A and fundus on patient 16 (Table 2)

per degree, and in individual eyes in 75 % in DpR1 and in 83 % in NDPR. The total pathological decrease in values of CS in all eyes was only in 41 % in DpR2, then in 60 % in DpR1, and the most pronounced was in NPDR at 68 %. In the case of deeper foveolas with a larger surface area of the FAZ, CS was without pathological values in 50 % in DpR2 and in 25 % in NPDR. Overall normal or borderline values of CS were recorded in all cycles per degree in eyes in 40 % in DpR1 and in only 32 % in NDPR. The lowest rate of affliction of CS without pathological values was represented by the finding in DpR2 in 59 % of individual eyes. Neither type 1 nor type 2 DpR was decisive for the decrease of CS in DpR, but rather the depth of the foveolas with regard to oxygenation of the photoreceptor cells, and the thus ensuing secondary capillary reactivity. A fundamental difference between both forms of DpR and NPDR in addition to the decrease of CS was the finding on OCT-A. DpR2 and NPDR mostly had a larger surface area of the FAZ, but in DpR2 this was the overall configuration of the fovea conditioned by the depth of the fovea and the overlap of capillaries into the FAZ due to worsened oxygenation of the retina. In NPDR the configuration was irregular due to loss of capillaries, which attested to more pronounced non-perfusion. Furthermore, in NPDR there was evident presence of MAs in the SVC and irregular dilation of capillaries in the DVC.

HbA1c: In a two-year observation period the development of the levels of HbA1c was assessed in two groups of patients, specifically 8 patients (DpR1 and NPDR) with a shallower foveola and smaller surface area of the FAZ. Its value was within the range of 45–80 mmol/mol, on average 66 mmol/mol. Only two patients had a Hb1Ac value under 60 mmol/mol within the given period. The second group comprised 10 patients (DpR2 and NPDR) with a deeper foveola and enlarged surface area of the FAZ, the HbA1c value was within the range of 47–117 mmol/mol, on average 64 mmol/mol. A total of four patients from this sub-group had a Hb1Ac value under 60 mmol/mol. This did not concern a cumulative evaluation of Hb1Ac within a period of at least 5 years. Furthermore, it was not possible to assess the evaluated cohort statistically due to the small number of subjects. Metabolic compensation was analogous on average for both groups. The included analysis of four T1DM patients evaluated two types of DpR and twice NPDR individually, with different depths of the foveolas and FAZ sizes.

Fig. 3 – DpR1: 21-year-old male diabetic patient in whom the onset of the metabolic disorder occurred at the age of 6 years, and DpR1 was manifested 3 years ago (patient no. 1– Table 1). The level of Hb1Ac within the period of duration of the two-year study fluctuated within the range of 52–62 mmol/mol. Documented finding in right eye – CS was pathological in 3 and 6 cpd. SD-OCT showed central thickness of 279 µm, average retinal thickness was 284 µm, from which it ensues that the patient had a very shallow foveola of -5 µm with the presence of hyperreflexive points. The FAZ was of a regular shape, with a surface area of 0.06 mm^2, in the SVC with minimal signs of non-perfusion, which was manifested also in the DVC with a practically disappeared FAZ. "Flecking" was visible on the fundus in the region of the foveola and its surrounding area.

Fig. 4 – DpR2: Young female diabetic patient with onset of T1DM at the age of 5 years and duration of the disease for 25 years, DpR was manifested at the age of 18 years (patient no. 20 – Table 1). H1Ac was within a minimal range around 50 mmol/mol (range 47–52 mmol/mol) in the last five years. Documented finding in right eye – CS was borderline in three cycles, except for 12 cpd, where it was reduced. SD-OCT showed a pronouncedly deepened fovea of -44 µm with central retinal thickness of 263 µm and average retinal thickness of 307 µm. HEs were visible on ophthalmoscopy, documented on a photograph of the ocular fundus. The FAZ was regularly rounded, with a surface area of 0.54 mm^2. SVC with minimal non-perfusion, but with overlapping capillaries into the FAZ. DVC regular with non-perfusion only in close proximity to the FAZ.

Fig. 5 - NPDR: 21-year-old female patient with onset of T1DM at the age of 3 years and NDPR manifested one year ago (patient no. 4 – Table 2). The level of Hb1Ac has worsened markedly in the last two years within a pathological range between 98–117 mmol/mol. Until the patient reached adult age Hb1Ac did not exceed the value of 70 mmol/mol due to parental supervision, and the finding was similar to DpR2. Documented finding in right eye – CS was reduced in three cycles, borderline only in 3 cpd. SD-OCT showed a pronouncedly deepened foveola of -74 µm (range between 220 µm central and 294 µm average retinal thickness) with thickened edges of the foveola, documented on photograph of ocular fundus, together with flecking. The FAZ was of a slightly irregular shape with overlapping capillaries on a surface area of 0.46 mm^2. The SVC was accompanied by irregular non-perfusion and isolated MAs. The DVC was of an irregular shape with pronounced non-perfusion around the FAZ and isolated dilated capillaries.

Fig. 6 – NPDR: Young male diabetic patient with onset of T1DM at the age of 10 years, duration of disease 17 years, in whom NDPR was diagnosed at the age of 24 years (patient no. 16 – Table 2), HbA1c in the last two years within the range of 60–77 mmol/mol. Documented finding in left eye – CS showed pathological values in all cycles. SD-OCT showed central retinal thickness of 310 µm, but average retinal thickness was 275 µm, from which it ensued that the depth of the foveola was +35 µm (a plus value was diagnosed in only one patient from the cohort), furthermore there was evident accumulation of fluid here. The FAZ was of a regular shape with a surface area of 0.06 mm^2 and presence of isolated MAs (SVC) and dilation of capillaries (DVC) with signs of non-perfusion in both vascular segments.

The above examples confirmed that an individual approach to adhering to a regimen was required for the development of ocular changes. A comparison of two female patients demonstrated that pronounced exceeding of the HbA1c norm within the course of two years led to a transition from DpR to NPDR, while conversely an image of DpR was maintained throughout 25 years of duration of T1DM thanks to long-term compensation. In two patients with analogous time factors (duration of T1DM and analogous onset of DpR, but unfortunately also NPDR), the development of ocular

changes was in connection with the level of Hb1Ac, since it is not possible to assume that the development of their surfaces differed markedly in the preceding period.

DISCUSSION

The prevalence of T1DM in childhood age is expected to increase from 94 000 in 2005 to 160 000 in 2020 [18]. In a Czech study, modelling identified two changes in the growth of T1DM: in 1995 the incidence accelerated, whereas in 2001 the increase in incidence slowed markedly [19]. The last extensive study on the increase of T1DM in children from 26 European countries, processed in 2019, deals with a 25-year period from 1989 to 2013. Unfortunately the Czech Republic (CZ), with an average increase of 4.7 % (within the range of 4.3 to 5 %), ranked in fifth place in the European Union (EU), with the largest increase recorded in Poland at 6.6 % and the lowest in Spain with only 0.5 %. The overall range of increase of T1DM on average in Europe was from 2.8 to 3.9 % [20]. In 2013 a total of 861 000 diabetic patients were recorded in CZ, representing 8.3 % of the population. Of these, 790 000 patients (91.7 %) suffered from type 2 diabetes (T2DM), and 61 000 patients (6.8 %) from T1DM. DR was detected in 103 000 patients [4]. An average increase of 60 000 new patients per year is expected, with 22 000 patients dying of diabetes annually. At present it is possible to assume that there are over a million patients in CZ with both types of this serious disease. From this it ensues that there is a society-wide necessity to introduce screening programmes for the prevention of diabetes, including DR. T1DM as an autoimmune disorder appears in childhood. As a result, its problems and ocular affliction are passed on into adulthood. A timely sign of DR, preceding an actual image of NPDR, is considered to be neurodegenerative processes [21, 22] within the framework of a reduction of plexiform fibres and ganglion cells [21], selective thinning of inner retinal fibres [22], defective function of the amacrine and bipolar cells [23] or conversely thickening of the plexiform and nuclear layer [22]. The detection of these conditions has been enabled by modern SD-OCT instruments working on the principle of three-dimensional patterns of the structure of the retina and its layers [24,25]. Peripapillary nerve fibres were reduced depending on the level of HbA1 in comparison with 7 % in pre-clinical DR [26].

Retinal neurodegeneration is present earlier in contrast with ophthalmoscopic detection of microcirculatory abnormalities. Neuroretinal damage triggers functional abnormalities such as reduction of colour perception, CS, adaptation to darkness [27], and provides a background for a pathological response in electrophysiological examinations [28]. The pathophysiology of diabetic changes is used as the basis in detecting initial changes. Hyperglycaemia is accompanied by oxidation stress and emergent pseudohypoxia, which may breach the cellular membrane of the vascular endothelium. Other theories refer to damage to pericytes by paradoxical glucose starvation, which leads to their apoptosis [3]. On the basis of this, a deficiency of nutrition of the photoreceptor and other retinal cells is assumed. This provides the background for a pathological response upon electrophysiological examinations and a reduction of contrast sensitivity. The interpretations and comparison of results of these methods are not uniform. Reduction of oscillatory potentials was accompanied with a decrease of contrast sensitivity in various stages of DR [29], and a decrease of amplitude of visual evoked potentials was recorded also in T1DM without DR, together with a reduction of CS [30], or abnormalities thereof before the onset of retinopathy, with normal VA [31].

A series of specialised ocular examination methods exist which specify a diagnosis of DR and DME, including assessment of the result of their treatment. Since 2016, the four basic diagnostic procedures in CZ have been: examination on a slit lamp, stereoscopic photography or digital stereophotography, fluorescein angiography (FAG), and OCT on the principle of low-coherence interferometry [4]. Other diagnostic procedures include: colour sensitivity, CS, electroretinogram (ERG), microperimetry and above all OCT-A. Individually or in combination they are capable of identifying the risk of onset of these ocular complications before their detection with the aid of photo documentation. We used a combination of examination methods in this study. An important component was also ophthalmoscopic examination, in which we recorded a change in the image of the central region, which we referred to as "flecking". We likened this symptom of map-like changes only in the macula to the image and term "retinal flecking", which relates to crystalline retinopathy associated with a disorder of the fat metabolism, and also simultaneously affects the periphery of the retina [32].

Studies on changes of CS in diabetic patients have been conducted abroad since the 1980s [33,34], and have determined that CS is more sensitive for the onset of diabetological changes of the retina than VA. CS demonstrated a pathological progression corresponding to worsening diabetic changes on the retina [35]. Changes of CS were detected in connection with laboratory changes in patients with T1DM, specifically a decrease of the CS score in microalbuminuria [36], and also in hyperglycaemia the CS score was together with visual disturbance, whereas VA was within the norm [37]. During the course of hypoglycaemia, CS also had a tendency to worsen [38]. Systematic observation of CS in child and adolescent patients was not frequent [28,36,39,40], but confirmed a decrease of CS in patients with T1DM with abnormal values in DR, in contrast with control groups. Information on the influencing of Hb1Ac in CS is variable. Decrease of Hb1Ac from pathological values was accompanied by a balancing of CS [28,41]. By contrast, no changes of CS conditioned by metabolic control of T1DM with the aid of Hb1Ac were demonstrated [9,39,42]. In CZ also, studies appeared at the end of the last century which determined a negative influence on CS by the factor of patient age of over 35 years and a longer duration of T1DM [42,43]. In patients with T1DM with or without DR, VA was not affected, whereas colour perception and mesopic foveal CS were significantly impaired in comparison with a control group [44]. The range of diagnoses in which it is possible to use this non-invasive and quick method of CS in ophthalmology is broad.

Neuritis of the optic nerve upon a background of multiple sclerosis always has reduced CS, even after the subsidence of an attack of the pathology, and is more sensitive in detecting changes than visual evoked potentials [45]. In the case of optic neuropathy, a decrease of CS helps identify incipient changes before a deterioration of vision [46]. CS may be influenced by acute change of refraction or formation of a cataract in T1DM. There is no evidence to support the theory of onset of "diabetic myopia", which would link it with metabolic dysregulation. Only in myopia is there a longer duration of diabetes, which prevents its onset [47]. The degree of transitional hyperopia is linked with the speed of correction of hyperglycaemia depending on the reduction of the glucose level in plasma [47,48]. The accumulated effect of hyperglycaemia is in direct correlation with a change of transparency of lenses in diabetics [49]. Fortunately taurine plays a significant role in the protection of the lens against the onset of cataract, serving for osmoregulation and at the same time acting as an antioxidant. The onset of cataract is influenced by an increase of sorbitol and water in the lenses both before and after the formation of the cataract [50]. We assessed changes of transparency of lenses in CS previously, but without demonstrating any fundamental influence [51]. Fortunately up to this time we have not yet recorded the onset of cataract in the second decade of life, which is always associated with a preceding deterioration of VA, hyperglycaemia and hyperopia [52,53].

From other of the above-stated examinations it ensues that despite the fact that colour perception and CS have similar characteristics, CS is markedly more sensitive and specific [54]. DR may accompany a yellow-blue defect of colour perception (tritanomaly) upon normal VA [55], above all upon a progression of DME with increasing age [56]. In our previous study we did not record any fundamental changes of colour perception in children and youth in comparison with its distribution within the population [7]. There was a significant decrease of b-wave ERG in implicit time in patients with incipient DR [57]. In patients with NPDR the increase in macular thickness, in addition to the prolonging of the implicit time of ERG, was accompanied also by a reduction of the amplitude and deterioration of VA [58]. Sensitivity of the retina in microperimetry was reduced upon increased central thickness of the foveola in NPDR, but from the perspective of contemporary individual modern methods of OCT no fundamental differences were determined within the framework of a microperimetric analysis [59,60].

OCT has been used regularly for over ten years for assessing the thickness of the macular region in cystoid macular edema for evaluation of the frequency of changes [61]. Assessment of actual retinal thickness has demonstrated a significant difference between the healthy population and patients with proliferative and non-proliferative form of retinopathy with the aid of a Zeiss-Humphrey instrument [62], in which retinal thickness was greater than in healthy subjects [61]. We confirmed this when there was no fundamental difference in already increased retinal thickness during the further course of the pathology in diabetics after 10 years of duration of the disease. The actual norms of macular cubature and central retinal thickness published in 2014 [10] can be taken to be reliable, since they were similar also to a later observation which stated cubature at 9.98 ±0.43 μm^3 and central retinal thickness at 280.67 ±12.79 µm in men and 276.63 ±11.61 µm in women [63]. In our study we did not assess this difference. SD-OCT is capable of demonstrating retinal thickening in the macular region with simultaneous reduction of CS before any clinical manifestations of DME [10,44]. SD-OCT in its cross-section enables the display of hyperreflexive spots, flecks in the inner plexiform layer, thus encroaching also into the layer of the ganglion cells and inner core layer. Upon progression they also affect the outer plexiform layer in connection with microglial activation, and their number increases [64]. We have already confirmed this finding of development in DpR, and an acoustic shadow was also frequently evident. Beyond the outer plexiform layer (Henle fibre layer) there follows the outer core layer, after which there are only photoreceptor cells [14]. After anti-VEGF therapy, DME was recorded in cases of its incomplete solution of their reduction, but without a significant correlation of VA [65].

Due to its non-invasiveness in certain indications, OCT-A may replace FAG, since unlike FAG it enables repeatability of the examination [17]. The size of the FAZ measured with the aid of FAG and OCT-A is comparable [66]. The diameter of the physiological circle of the FAZ is highly variable, within the range of 250–600 µm [14], which represents a surface area from 0.052 to 0.283 mm2. In a control study [10] we determined a larger diameter of the circle of the FAZ, specifically 700 µm. Repeated measurements of the FAZ are comparable, and do not depend on the instrument used [67] or on whether the measurement was conducted automatically or manually [68,69], as we conducted them ourselves. In CZ four types of instruments [17] are used in the assessment of vascular density, and there is no significant difference between them. In the evaluation of the number of distinguishable bifurcations of vessels there is a difference, but not of statistical significance [70]. Assessment of the quantitative blood flow through the retina is decisive for the OCT-A image [71]. In the healthy population and in diabetic patients without DR it is similar, whereas by contrast, in mild form of NDPR the flow in the arteries is retarded, and is significantly reduced in the veins [72]. Retardation of through flow causes only 40 % visibility of MA in OCT-A, in contrast with FAG [73], though OCT-A is nevertheless of better quality in visualisation of MA than SD-OCT [74], since it concerns pure visualisation and more precise comparison [75]. OCT-A in patients with T1DM before the onset of DR shows a reduction in the overall density of the capillaries [76] and frequent microvascular changes [77,78] primarily in the parafoveal region [79,80], which we also observed. We also recorded thinning of the capillary the SVC [81] and in the DVC [80], which confirms quantification of perfusion in each layer [82]. Further thinning and loss of the capillary network continues in the progression to NPDR [83], which we also confirmed. Worsening of DR is also accompanied by further changes in the DVC [84], an example of which is our observation of dilation of the capillaries in this vascular segment.

A significant difference in retinal microvasculature is evident between healthy subjects and patients with T1DM, and may detect changes on the retina earlier than a biomicroscopic finding [85]. In addition to MA it also detects retinal neovascularisation, intraretinal microvascular abnormalities and a different pattern of arteries or veins [86]. The most recent evaluation of the ganglion cell layer detected focal losses which may increase over time, and which could serve as a timely index of neuronal damage in patients with T1DM in the case of an apparent absence of symptoms of DR [87]. This fact again confirms the primary role of neurodegenerative changes in the progression of ocular affliction.

In addition to retinal changes, changes are also described in the choroid in diabetic patients, though we did not deal with this issue within the framework of our own study due to the limitations of the instrument equipment. Choroidal thickness generally and in the outer part is thinner in patients with NPDR in comparison with the healthy population [88], which applies also for patients without symptoms of DR, and furthermore it may be functionally different [89]. This factor is in direct correlation with the evolution of the underlying pathology [90], and thinning is also manifested in the region of the fovea [91]. The picture of choroidal changes includes increase of vascular tortuosity, neovascularisation, and also dilation or conversely obstruction with outages manifested in non-perfusion [92]. Choroidal thinning is prevented by maintaining the level of HbA1c beneath 7 % [93].

A factor which cannot be overlooked for the onset of ocular complications of T1DM is the length of its duration. The Wisconsin studies demonstrated that a range of risk factors exist which fundamentally and significantly support the development of DR and the increase of DME. These include high blood pressure, proteinuria, smoking, high Body Mass Index and prevalence in the male sex [94,95,96]. Weight gain during the course of childhood is a fundamental risk factor for the triggering of T1DM [97]. Variability of HbA1c presents a further fundamental and independent factor in connection with the development of DR [96,98] and nephropathy [99]. A variable level of Hb1Ac is also a risk, since a high baseline level of Hb1Ac and its subsequent marked reduction is a risk factor for the thickening of the macular region [100]. By contrast, changes in daily levels of glucose did not demonstrate a significant dependency on the development of microvascular complications [27,101]. Though we did not engage in a cumulative evaluation of HbA1c, we nevertheless documented the fundamental importance of metabolic compensation also in the prevention of the progression of DR. These relationships [98,102] have long been documented by several studies by the DCCT (Diabetes Control and Complication Trial) and EDIC (Epidemiology Diabetes Intervention and Complication).

CONCLUSION

We have demonstrated that an intermediate degree exists between a physiological finding on the retina and NPDR, namely diabetic pre-retinopathy, which can be considered a pre-clinical form of DR. It is divided into an image with a deeper foveola, more extensive FAZ with a lesser decrease of CS and a condition with a shallow foveola, a narrower FAZ with a more pronounced decrease of SC. At the same time further changes of microvascularity (OCT-A) were recorded, such as disorders in the sense of non-perfusion in the central part of the retina of varying degrees. This finding differs significantly from changes in already triggered NPDR, in which a fundamental decrease of CS was determined, as well as significant disorders of non-perfusion and mainly the presence of microaneurysms.

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