AUTOMATIC RETINAL OXIMETRY IN PATIENTS WITH DIABETIC RETINOPATHY

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SUMMARY

Aim: To determine if oxygen saturation in retinal vessels depends on the degree of diabetic retinopathy.

Material and methods: A prospective study comparing values of oxygen saturation in retinal arteries, veins, and arterio – venous (A-V) difference in healthy persons and in patients with diagnosed diabetes. The study included 114 eyes of 76 patients with diagnosed diabetes, and 57 eyes of 57 patients without diabetes as a control group.

Results: The average retinal arterial saturation in patients without diabetes was 96.5 ± 2.6 %, and increased in patients with severe non-proliferative or proliferative diabetic retinopathy to 100.5 ± 5.6 %. The average venous saturation in patients without diabetes was 62.3 ± 7.4 % and increased to 74,0 ± 7.2 % in patients with severe non-proliferative diabetic retinopathy.

Conclusion: In patients with diabetic retinopathy, we confirmed the increase of hemoglobin oxygen saturation as in the arterial as in the venous blood in retinal vessels; and significant decrease of arterio-venous difference according to the severity of diabetic involvement was confirmed as well.

Key words: automatic retinal oxymetry, diabetic retinopathy, oxygen saturation, Oxymap

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INTRODUCTION

The dramatic growth in the incidence of diabetes in recent years has been accompanied by an increase in the number of patients with diabetic retinopathy. Despite substantial advances in the treatment of this pathology by means of systemic control, laser therapy, anti-VEGF preparations and vitrectomy, it remains one of the most common causes of blindness in industrially developed countries. Furthermore, at present the incidence of diabetic retinopathy is rising dramatically in developing countries. (18)

Diabetic retinopathy is characterised by damage to the retinal capillaries (13). The basal membranes of the capillaries thicken (16), some capillaries are obliterated and others become wider (3,14), forming microaneurysms. All of these changes occur upon a background of a rise in the level of glucose in the bloodstream. The precise biochemical processes are complex and are not entirely known to date. The consequence of damage to capillaries is impaired blood distribution, which may lead to hypoxia of the retinal tissue.

Hypoxia of the retina in diabetic retinopathy has been confirmed on a cat model (15) with the aid of oxygen-sensitive retinal electrodes. With regard to the invasiveness of the method of measurement, for a long time it was not possible to confirm these measurements in human patients.

Since 2006 non-invasive examination of oxygen saturation in large retinal blood vessels has been possible with the aid of automatic retinal oximetry (Oxymap ehf. Reykjavik, Iceland). (7)

Automatic retinal oximetry

The dual non-invasive retinal oximeter T1 (Oxymap ehf. Reykjavik, Iceland) is composed of two digital cameras, a special optical adapter, an image distributor and two narrow band filters. The entire device is connected to a standard fundus camera (Topcon, TRC – 50 DX, Topcon corp. Tokyo, Japan) (fig. 1), via which two images of the retina are obtained with different wavelengths – 570nm and 600nm. Special software (Oxymap analyzer software 2.2.1, version 3847; Oxymap ehf.) measures the luminosity of the selected points on the obtained images on both wavelengths. The points of measurement are placed by the software on large blood vessels and the retinal background along the blood vessels.

The light absorbance of the blood vessel is influenced by the absorbance (colour) of the blood inside the vessel, whereas the colour of the retinal background is relatively constant and not markedly influenced. In this manner it is possible to describe the optical density (OD) of the blood vessel, for which the following applies:

$$OD = log (I_0/I),$$

in which I0 is absorbance outside of the blood vessel (on the retinal background) and I is absorbance of the blood vessel.

The optical density of the blood vessel is markedly influenced by saturation of oxygen upon production of an image by light with a wavelength of 600nm (arteries are substantially darker than veins), but not at a referential wavelength of 570nm (arteries and veins have a virtually identical appearance) (see fig. 2).

The proportion of optical densities at wavelengths of 600nm and 570nm determines the “optical density ratio” (ODR):

$$ODR = OD600/OD570$$

ODR therefore has an inverse and approximately linear dependency upon oxygen saturation:

Saturation O2 % = a+b x ODR,

in which a, b are constants.

For calibration of the Oxymap T1 instrument, the values used were OD600 and OD570, obtained from measurement on healthy patients. These values were compared with measurements on an already calibrated instrument, which was performed by Schwitser et al. (17). The automatic software therefore attributes a value of oxygen saturation to each point, expressed with the aid of a colour scale, as can be seen in fig. 3.

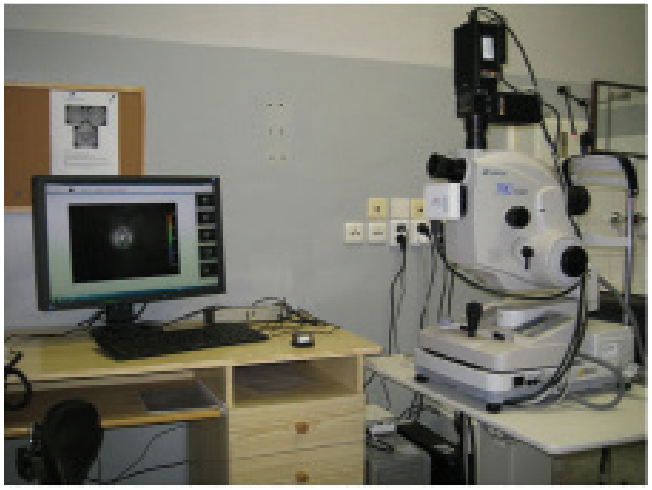

Fig. 1 Dual non-invasive retinal oximeter T1 (Oxymap ehf. Reykjavik, Iceland) connected to standard fundus camera (Topcon, TRC – 50 DX, Topcon corp. Tokyo, Japan)

AIM OF STUDY

To determine whether oxygen saturation in retinal blood vessels depends on the degree of progression of diabetic retinopathy.

METHOD

Prospective study comparing values of oxygen saturation in retinal arteries, veins and also the arteriovenous (A-V) difference in healthy individuals and in patients with diagnosed diabetes. In all patients measurement of retinal oximetry was performed with the aid of the Oxymap instrument (Oxymap ehf. Reykjavik, Iceland). A detailed description and the principle of the device is provided above in the introduction to this study. Examination of the patients with the aid of automatic retinal oximetry on the Oxymap instrument was approved by the Ethical Commission of the Faculty of Medicine of Palacký University and the University Hospital in Olomouc.

We used standardised conditions for the measurement of retinal oximetry. In all cases the measurement was performed in a dark room in order to eliminate the influence of light on the result (8). Fundus photography was performed in a minimum of two specimens, of which that with the highest quality for the performance of an analysis was selected. Fundus photography was performed in a width of 50° and was centred to the temporal edge of the optic nerve. The power of the flash (illumination of photograph upon exposure) was set at 50 Ws for all photographs.

The recommendations for the last version of the analytical protocol from November 2013 were used for the analysis of oxygen saturation (Oxymap protocol for acquisition and analysis of Oxymap T1 oximetry images, version November 21, 2013; Oxymap Inc.)

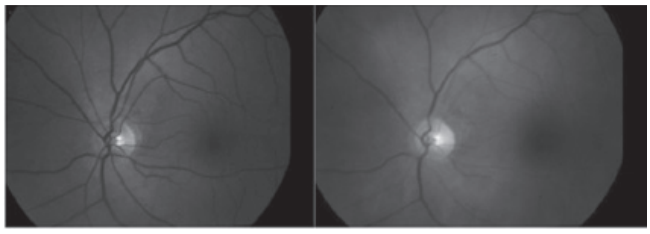

Fig. 2 Comparison of image of fundus of one eye in same patient: right image with wavelength of 600nm (arteries are substantially darker than veins) and left with wavelength of 570nm (arteries and veins have virtually identical appearance).

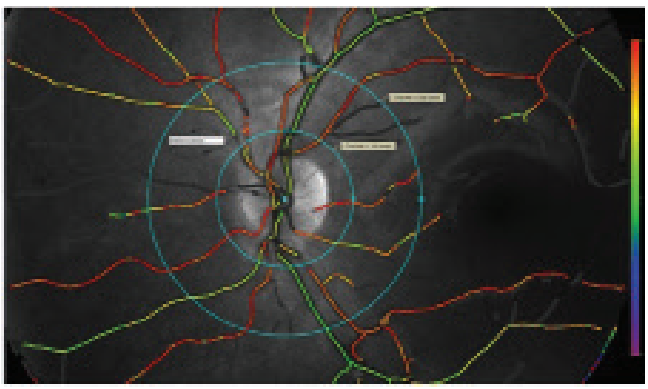

Fig. 3 Ocular fundus of patient with display of staturation of blood in retinal blood vessels with the aid of a colour scale. The image shows the measurement zone, which is bordered by two concentric rings with a size of 1.5 PD and 3 PD with the centre of both rings in the centre of the papilla.

Only retinal arteries and veins with a diameter larger than 8 pixels were included in the analysis. Measurement was performed on blood vessels in the "measurement zone". This area is bordered by two concentric rings with a size of 1.5 PD and 3 PD, with the centre of both rings in the centre of the papilla (fig. 3).

Overall average saturation was calculated as a sum of the oxygen saturations of all arteries (or veins) supplied by the fourth power of their diameters, with subsequent proportion of the sum of the fourth power of the diameters of all arteries or veins (4, 21). The following formula applies for the case of measurement of eight blood vessels:

Average oxygen saturation

$$= \frac{S_1 * d_1^4 + S_2 * d_2^4 + S_3 * d_3^4 + S_4 * d_4^4 + S_5 * d_5^4 + S_6 * d_6^4 + S_7 * d_7^4 + S_8 * d_8^4}{d_1^4 + d_2^4 + d_3^4 + d_4^4 + d_5^4 + d_6^4 + d_7^4 + d_8^4}$$

In which:

S = saturation of nth vascular segment *d = diameter* of nth vascular segment Arteriovenous difference (A-V difference) was calculated as the difference between average arterial and venous oxygen saturation (21).

Statistical method: Shapiro-Wilk normality tests were used for testing the normal distribution of the examined quantity. Normal distribution was demonstrated in all compared groups, as a result an ANOVA dispersion analysis was used for comparison with subsequent Bonferroni post hoc multiple comparison tests. Unless stated otherwise, the data is presented as the average ± standard deviation. The boundary of statistical significance was set at the value of p = 0.05. All calculations of the statistical processing were performed using the program SPSS v.15 (SPSS Inc. Chicago, USA).

COHORT OF PATIENTS

The study incorporated 114 eyes of 76 patients with diagnosed diabetes, and 57 eyes of 57 non-diabetic patients, who served as a control group. The group of patients with diabetes was further divided into 4 subgroups according to the clinical finding based on the classification of the American Academy of Ophthalmology (AAO) (22). The first subgroup comprised patients with diabetes without signs of diabetic retinopathy, the second subgroup consisted of patients with mild nonproliferative diabetic retinopathy, the third with medium advanced diabetic retinopathy and the fourth with advanced nonproliferative or proliferative diabetic retinopathy. The presence (or absence) of diabetic macular edema was not taken into consideration in this study upon classification of patients into subgroups. The patients were taken into the study progressively in the period from September 2013 to December 2014. We obtained the information about the diagnosis, type, period of duration of diabetes and treatment from the report of the attending diabetologist. A sample of the current level of glycated haemoglobin HbA1c was taken from all diabetic patients. A condition for entry into the study was transparent ocular media, enabling us to obtain images of the ocular fundus of sufficient quality for evaluation by Oxymap. Conditions following undertaken LPC (laser photocoagulation) or PPV (pars plana vitrectomy) were exclusion criteria for entry into the study due to their influence on the precision of the results of automatic retinal oximetry (11, 19). All the demographic data about the patients is summarised in table no. 1.

RESULTS

Average retinal arterial saturation in our study in the patients without diabetes was 96.5 ± 2.6%; in the patients with diabetes without visible signs of diabetic retinopathy it was 96.5 ± 3.2%; in the group with mild diabetic retinopathy 96.7 ± 4.6%; in the group with medium severe diabetic retinopathy 97.8 ± 4.6% and in patients with severe nonproliferative or proliferative diabetic retinopathy arterial saturation was 100.5 ± 5.6%.

Average retinal venous saturation in the non-diabetic patients was 62.3 ± 7.4%; in diabetics without diabetic retinopathy 66.3 ± 6.3%; in the group with mild diabetic retinopathy 67.9 ± 7.2%; in the group with medium severe nonproliferative diabetic retinopathy 69.9 ± 6.7% and in the patients with severe nonproliferative or proliferative diabetic retinopathy the average venous saturation was 74 ± 7.2%.

The arteriovenous difference in the healthy patients was on average 34.3 ± 7.2%. In the diabetic patients without retinopathy the average A-V difference was 30.2 ± 4.9%; in the patients with mild diabetic retinopathy 28.8 ± 8.2%; in the patients with medium severe diabetic retinopathy 27.9 ± 5.8%, and 26.5 ± 7,8% in the patients with severe nonproliferative or proliferative diabetic retinopathy. Tables 2, 3 and 4 show box graphs of measured differences. At the same time, statistically significant differences with the value of p are shown.

DISCUSSION

Our results independently confirm the already several times published fact that in patients with diabetic retinopathy there is an increase in the values of oxygen saturation mainly in the venous system in comparison with healthy patients (6, 9, 11). At the same time, our study indicates the same previous results of dependency of the degree of retinal affliction by diabetes on changes in saturation (10).

From a general perspective, oxygen saturation in retinal blood vessels is dependent upon the speed of blood flow (time of capillary through flow) and the consumption of oxygen in the peripheral tissue – retina. At least three known mechanisms contribute to changes of oxygen saturation in

Table 1 Demographic data on patients

	without DM (n=57)	*DM without DR (n=34)*	*NDR mild (n=25)*	*NDR medium (n=28)*	*NDR severe + PDR (n=27)*
age (years)	57,3 ± 8	46,2 ± 16	52,8 ± 14	50,9 ± 17	53,6 ± 15
sex M/F	24/33	16/18	12/13	18/10	21/16
number of IOL	14	8	13	11	14
level of HbA1c (mmol/mol)	-	65,9 ± 15,9	66,1 ± 17,2	66,3 ± 16,1	68,5 ± 21,7
period of duration of DM (years)	-	11 ± 8,5	16,3 ± 9,2	17,1 ± 8,8	21,3 ± 7,6
ratio of DM1/DM2	-	18/16	10/15	11/17	9/18

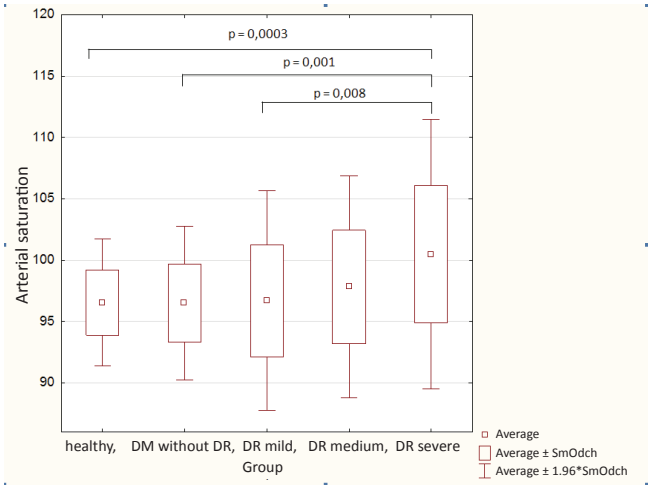

Table 2 Box graphs with values of average arterial saturation in indivual stages of diabetic retinopathy. Statistically significant differences are presented with the relevant value p.

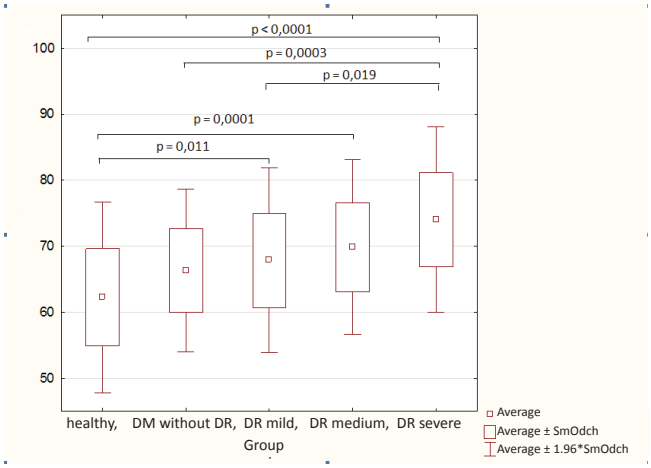

Table 3 Box graphs with values of average venous saturation in indivual stages of diabetic retinopathy. Statistically significant differences are presented with the relevant value p.

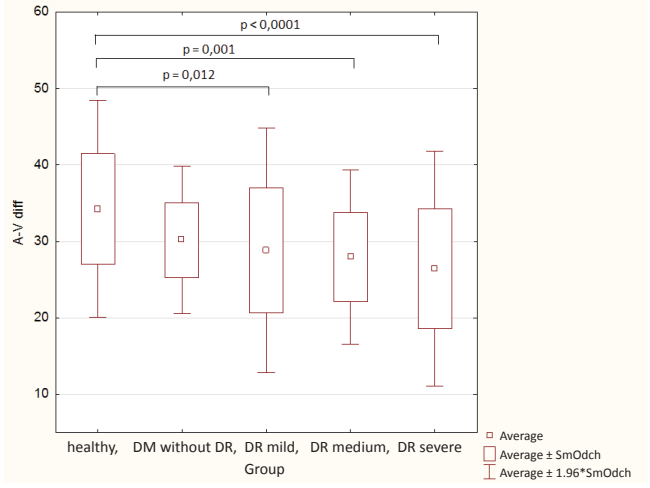

Table 4 Box graphs with values of average A-V difference in indivual stages of diabetic retinopathy. Statistically significant differences are presented with the relevant value p.

diabetics: 1. capillary nonperfusion and shunting of certain areas of the retina, 2. thickening of capillary walls and 3. higher affinity of haemoglobin for oxygen in diabetics.

Cogan and Kuwabara (3, 14) demonstrated that in diabetic patients a closure of certain capillaries takes place, whilst others are dilated. In diabetic patients we demonstrate nonperfusion and dilation of retinal capillaries and arterioles in various regions of the retina with the aid of fluorescence angiography. The result of these changes is that a large amount of blood flows through the dilated capillaries at a high speed from the arteries into the veins, without a sufficient amount of oxygen being absorbed into the target tissue (retina). This mechanism leads to an increase of saturation in the venous blood.

A further mechanism which could contribute to changes in the resulting oxygen saturation of retinal blood vessels is the thickening of capillary walls in diabetics, which is unequivocally demonstrated (1,16). This thickening is responsible for a deterioration of the diffusion of oxygen from the blood into the retinal tissue, and thereby contributes to a larger amount of oxygen in the venous blood.

A third mechanism which may contribute to an overall increase in saturation in the retinal blood vessels is an increase in the affinity of haemoglobin for oxygen, together with an increase in haematocrit in diabetic patients (5,12). The higher affinity of haemoglobin for oxygen and the larger number of red blood cells could explain the higher values of saturation in the arterial and venous bloodstream.

The precise differentiation of the proportion of individual mechanisms in the resulting retinal saturation is so far unclear. At the same time it is probably that as yet unknown mechanisms exist, which contribute to changes in retinal saturation in the retinal bloodstream. In future it shall be necessary to perform further studies in order to reach a deeper understanding of all the mechanisms.

A significant question remains concerning the connection between diabetic macular edema (DME) and the resulting oxygen saturation. In all the studies published to date on this theme, different saturation has been demonstrated in retinal blood vessels in patients with DME, and a separate subgroup has been created for patients with DME (6, 9, 10). DME is a separate complication of diabetic retinopathy and may be present in all its stages (2). In our study we decided not to include patients with DME. From our perspective the creation of such a subgroup is not a good approach. In such a small cohort it is not possible to demonstrate unequivocally a causal connection between the presence of diabetic macular edema and changes of oxygen saturation in the retinal blood vessels. In order to demonstrate the aforementioned dependency it would be appropriate to conduct a large study comparing groups of patients with the same satage of affliction within the framework of diabetic retinopathy, with and without diabetic macular edema.

A weakness of the cohort remains the common evaluation of phakic and artephakic patients. The study was prepared at a time before the processing of the annual results on the evaluation of the effect of PPV on oxygen saturation, in which we described the influence of lens opacities

(cataracts) on the measurement of oxygen saturation with the aid of automatic retinal oximetry (20). According to the results of the aforementioned study, in patients with lens opacification an artificial increase in the values of saturation occurs. It is nevertheless a fact that this disadvantage is manifested mainly in the observation of these values over time in a single patient. In the case of a one-off measurement only an error of the absolute value of the number can occur due to this influence, though the trend should be preserved upon maintenance of a similar ratio of phakic and artephakic patients.

A further weakness of our cohort of patients is the control group without diabetic retinopathy, which is not comparable in terms of age with the other groups (age-matched controls).

An advantage of the study is the prospective design and equal representation of patients in all the observed groups in comparison with the study by Jørgensen et al. (10).

CONCLUSION

In patients with diabetic retinopathy we demonstrated an increase in oxygen saturation of haemoglobin both in arterial and in venous retinal blood, and at the same time we demonstrated a significant reduction of the arteriovenous difference depending on the severity of the diabetic affliction. The results of the study complement and confirm previously published hypotheses which refer to changes of the oxygen metabolism upon the development of diabetic retinopathy. The results of the study of changes in retinal saturation could contribute in future to a deeper understanding of the pathophysiological nature of diabetic retinopathy and thereby improve the prevention and treatment of this serious disease.

LITERATURE

1. **Ashton N:** Vascular basement membrane changes in diabetic retinopathy. Montgomery lecture 1973. Br J Ophthalmol, 58; 1974: 344–366.
2. **Boguszaková J, Doležalová J, Gajdošíková Z:** Diabetická makulopatie. Čes Oftalmol, 50; 1994: Suppl. S12–8.
3. **Cogan DG, Kuwabara T:** Capillary Shunts in the Pathogenesis of Diabetic Retinopathy. Diabetes, 12; 1963: 293–300.
4. **Feke GT, Tagawa H, Deupree DM et al.:** Blood flow in the normal human retina. Invest Ophthalmol Vis Sci, 30(1); 1989: 58–65.
5. **Graham JJ., Ryall RG, & Wise PH:** Glycosylated haemoglobin and relative polycythaemia in diabetes mellitus. Diabetologia, 18(3); 1980: 205–207.
6. **Hammer M, Vilser W, Riemer T et al.:** Diabetic patients with retinopathy show increased retinal venous oxygen saturation. Graefes Arch Clin Exp Ophthalmol, 247(8); 2009: 1025–30.
7. **Hardarson SH, Harris A, Karlsson RA et al.:** Automatic retinal oximetry. Invest Ophthalmol Vis Sci., 47(11); 2006: 5011–6.
8. **Hardarson SH, Basit S, Jonsdottir TE et al.:** Oxygen saturation in human retinal vessels is higher in dark than in light. Invest Ophthalmol Vis Sci., 50(5); 2009: 2308–11.
9. **Hardarson SH, Stefánsson E.:** Retinal oxygen saturation is altered in diabetic retinopathy. Br J Ophthalmol., 96(4); 2012: 560–3.
10. **Jørgensen CM, Hardarson SH, Bek T.:** The oxygen saturation in retinal vessels from diabetic patients depends on the severity and type of vision-threatening retinopathy. Acta Ophthalmol, 92(1); 2014: 34–39.
11. **Jørgensen CM, Bek T.:** Increasing oxygen saturation in larger retinal vessels after photocoagulation for diabetic retinopathy. Invest Ophthalmol Vis Sci, 55(8); 2014: 5365–5369.
12. **Kennedy L, Baynes JW.:** Non-enzymatic glycosylation and the chronic complications of diabetes: an overview. Diabetologia, 26(2); 1984: 93–98.
13. **Kohner EM.:** Diabetic retinopathy. BMJ, 307; 1993: 1195–1199.
14. **Kuwabara T, Cogan DG.:** Retinal vascular patterns. VI. Mural cells of the retinal capillaries. Arch Ophthalmol, 69; 1963: 492–502.
15. **Linsenmeier RA, Braun RD, McRipley MA et al.:** Retinal hypoxia in long-term diabetic cats. Invest Ophthalmol Vis Sci, 39(9); 1998: 1647–57.
16. **Roy S, Ha J, Trudeau K, Beglova E.:** Vascular Basement Membrane Thickening in Diabetic Retinopathy. Curr Eye Res, 35; 2010: 1045–1056.
17. **Schweitzer D, Leistritz L, Hammer M et al.:** Calibration-free measurement of the oxygen saturation in human retinal vessels. Ophthalmic Technologies V. Proc SPI, 2393; 1995: 210–218.
18. **Sosna T.,** Švancarová **R., Netuková M.:** Diabetická retinopatie – rizikové faktory, prevence a terapie. Čes a slov Oftal, 66(5); 2010: 195–203.
19. **Šín M.,** Šínová **I., Chrapek O. et al.:** The effect of pars plan vitrectomy on oxygen saturation in retinal vessels - a pilot study. Acta Ophthalmol, 92(4); 2014: 328–332.
20. **Šín M., Chrapek O.,** Šínová **I. et al.:** The effect of pars plana vitrectomy and nuclear cataract on oxygen saturation in retinal vessels, diabetic and non-diabetic patients compared. Acta Ophthalmol, 94(1); 2016: 41–47.
21. **Traustason S, Jensen AS, Arvidsson HS et al.:** Retinal oxygen saturation in patients with systemic hypoxemia. Invest Ophthalmol Vis Sci, 52; 2011: 5064–5067.
22. **Wilkinson CP, Ferris FL, Klein RE et al.:** Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology, 110(9); 2003: 1677–1682.