Experimental Studies

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Morphometric analysis of retinal structural components in Wistar rats in experimental diabetes mellitus

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Purpose: *To perform morphometric analysis of retinal structural components in Wistar rats in experimental diabetes mellitus (DM) in an attempt to digitize possible retinal neurodegenerative changes.*

Material and Methods: *Eight histological globe preparations from Wistar rats were retrospectively reviewed. Of these, five were from Wistar rats with experimental DM, and three, from healthy controls. Total retinal thickness was measured and thicknesses of the following retinal layers were measured: the photoreceptor layer (PRL), outer nuclear layer (ONL), outer retinal layer (ORL), inner nuclear layer (INL), ganglion cell layer (GCL), and nerve fiber layer (NFL). The numbers of neural-cell rows in both nuclear layers were calculated visually.*

Results: *The mean total thickness of the retina was 252.9 ± 8.77 µm for healthy controls and 262.1 ± 8.51 µm for diabetic animals. In controls and diabetic animals, the mean thicknesses for particular retinal layers were as follows: PRL, 75.5 ± 4.14 µm and 74.0 ± 3.85 µm, respectively; ONL, 61.8 ± 3.04 µm and 66.1 ± 4.12 µm, respectively; ORL, 11.6 ± 0.72 µm and 12.4 ± 0.64 µm, respectively; INL, 33.1 ± 1.74 µm and 33.6 ± 1.75 µm, respectively; IRL, 47.2 ± 2.77 µm and 47.9 ± 2.39 µm, respectively; and GCL plus NFL, 23.7 ± 1.44 µm and 28.1 ± 2.57 µm, respectively. In addition, the numbers of neural-cell rows in the ONL were 11.3 ± 0.50, and 11.7 ± 0.59, respectively, and in the INL, 4.6 ± 0.26, µm and 4.7 ± 0.17, respectively. There was no statistically significant difference in thicknesses of retinal layers or numbers of neural-cell rows in the INL and ONL of the retina between normal and diabetic rats.*

Conclusion: *For Wistar rats with diabetes duration of 3 months, microscopic images of the retina and calculations of thicknesses of individual retinal layers and numbers of neural-cell rows in retinal nuclear layers provided no indication of neurodegenerative changes at this time point.*

Keywords:

diabetic retinopathy, diabetic mellitus, morphometry, experiment, neurodegeneration

Introduction

Diabetic retinopathy (DR) is a major complication of diabetes mellitus (DM). DM has become a non-infectious pandemic [1] and is the fourth cause of death globally [2], so it is not surprising that ophthalmologists often have to deal with DR patients. There are approximately 93 million people with DR, 17 million with proliferative DR, and 28 million with vision-threatening DR worldwide [3]. It is important that, in patients with type 1 DM, DR can develop as early as 3-5 years after the onset of diabetes [4]. Of an estimated 285 million people with DM worldwide, 93 million (34.6%) have signs of DR [5]. The number of individuals with DM was projected to increase to over 500 million by 2025, with about 90% attributed to type 2 DM [6], and further to about 600 million by 2035, with an increase in the number of patients with DR [7, 8, 9]. Individuals with diabetes are 25 times more likely to become blind than persons in the general population [10]. Proliferative diabetic retinopathy is a major cause of visual loss in diabetic patients and is characterized by neovascularization) that occurs at the vitreoretinal interface and in the vitreous. It can cause vitreous hemorrhage, neovascular glaucoma and tractional retinal detachment which can result in visual loss [10]. Diabetic macular edema is a relatively late manifestation of DR and also can cause blindness [11]. The risk of DR is increased in the presence of diabetic nephropathy and lower limb angiopathy [9]. Because "diabetes is still a major cause of blindness in adults older than 65 years of age", DR is a challenge for ophthalmologists [12].

Methods of treatment for DR are developed on laboratory animals. Since DR initially affects not the retinal

vasculature but neural retinal cells [13], determining the morphometric parameters of retinal components in various species of laboratory animals will allow identifying the species with the most apparent signs of retinal neurodegeneration in DR, which will be feasible to use in experimental studies aimed at the development of methods for DR treatment and prevention.

The purpose of the study was to perform morphometric analysis of retinal structural components in Wistar rats in experimental DM in an attempt to digitize possible retinal neurodegenerative changes.

Material and Methods

Eight archive histological globe preparations from Wistar rats of Pathological Anatomy Laboratory, SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine", were retrospectively reviewed. Of these, five were from Wistar rats with experimental DM, and three, from healthy control animals. Neonatal streptozotocin (STZ)-induced diabetes model was used. STZ (100 mg/kg body weight) was injected intraperitoneally into two-week rats for this purpose. Three months after initiation of diabetes, following euthanasia, globes were enucleated, paraffin embedded, and 5-μm sections were obtained and stained with hematoxylin and eosin. To perform morphometric calculations, we employed the classical histological methodology with the use of eyepiece micrometer. The actual size of each division of the eyepiece micrometer was determined with the help of the object micrometer at the specified magnification (objective magnification, x40, eyepiece magnification, x7; and objective magnification, x10, eyepiece magnification, x16) of the Laboval 4 microscope. Total retinal thickness was measured in micrometers, and thicknesses of the following layers were measured in the neurosensory retina: the photoreceptor layer (PRL), outer nuclear layer (ONL), outer retinal layer (ORL), inner nuclear layer (INL), ganglion cell layer (GCL), and nerve fiber layer (NFL). The numbers of neural-cell rows in both nuclear layers were calculated visually. All these characteristics were compared with those typical for the retinae of three normal Wistar rats of the same age (controls).

JASP G*Power 3.1 software was used for the analysis of measurement data. Mean and standard deviation (SD) values were calculated for each sample. The Student t-test was used to determine differences between mean values. P-values less than 0.05 were considered significant. In addition, the percentage contribution of each retinal layer to the full thickness of the retina was calculated and compared among the two groups. Morphometric measurement data are presented as mean (SD), whereas errors of mean for thicknesses of separate retinal layers are presented in the table.

Results

All retinal layers can be seen both in the retinae in untreated diabetic rats and normal rats (Figures 1-5).

Hematoxylin and eosin staining showed no apparent neurodegenerative changes in the retina in diabetic rats. However, individual retinal neuronal cells exhibited lysis, or vacuoles of various sizes, shapes and numbers developed in retinal cells due to edema. This apparent vacuolization (likely, hydropic vacuolization) of the GCL and NFL was typical for diabetic animals. Retinal vacuolization in diabetic animals can be seen in Figs 2-5, but most apparently, in Fig. 3. Diabetic Wistar rats showed no other structural neuroretinal abnormalities and normal choroidal structure.

We found that the Wistar rat retina has some special features such as (a) some waviness of the ONL surface towards the PRL due to the heterogeneous thickness of the ONL (Figs. 1-3); the presence of blood vessels penetrating

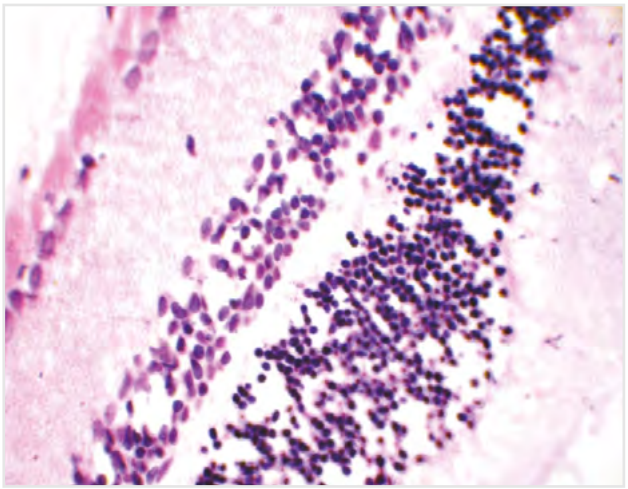

Fig. 1. Neural retina of a healthy control Wistar rat. Note the outer nuclear layer is profoundly heterogeneous in thickness. Hematoxylin and eosin staining. Objective magnification, x40; eyepiece magnification, x7.

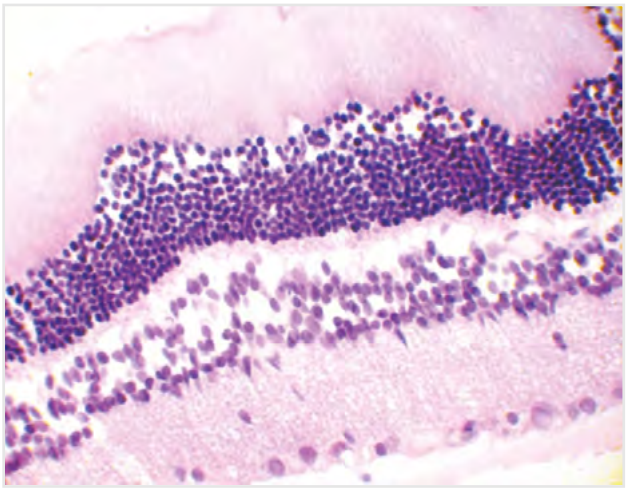

Fig. 2. Neural retina of a Wistar rat with 3-month diabetes duration. The inner and outer nuclear layers contain as much as 7 and 15 neural-cell rows, respectively. Hematoxylin and eosin staining. Objective magnification, x40; eyepiece magnification, x7.

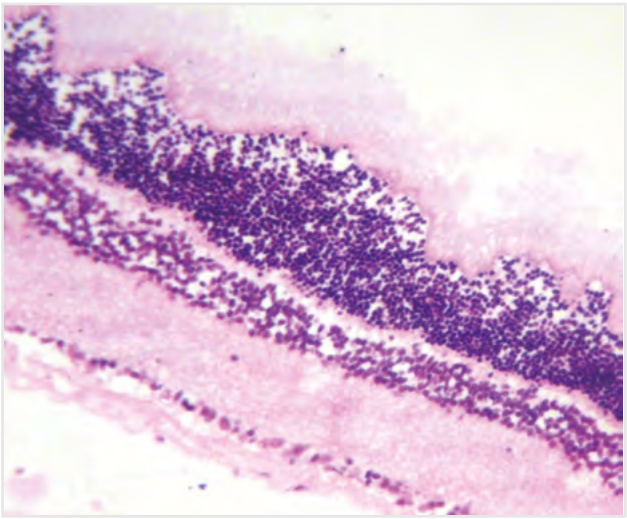

Fig. 3. Neural retina of a Wistar rat with 3-month diabetes duration. Note apparent edematous vacuolization of the ganglion cell layer and nerve fiber layer (arrows). Hematoxylin and eosin staining. Objective magnification, x10; eyepiece magnification, x16.

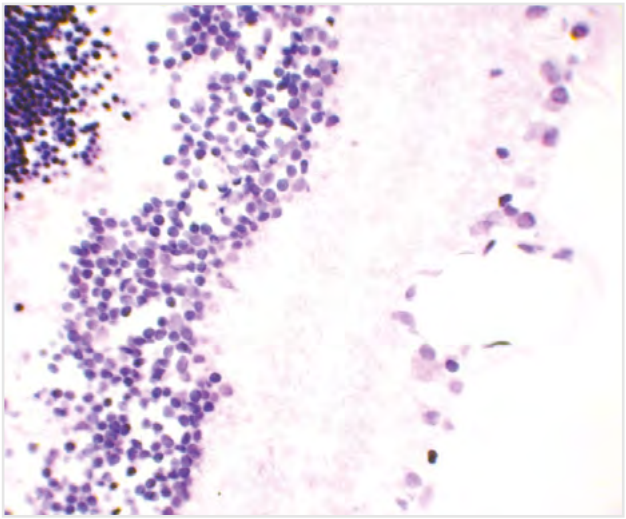

Fig. 4. Neural retina of a Wistar rat with 3-month diabetes duration. Note a blood vessel (an ectatic venule) in the ganglion cell layer and nerve fiber layer (arrow). Hematoxylin and eosin staining. Objective magnification, x40; eyepiece magnification, x7.

several layers including the INL (Fig. 4); and the absence of pigment in RPE cells because Wistar rats are albino.

Table 1 compares the group of diabetic animals with the group of normal control animals with regard to (a) thicknesses of particular retinal layers and (b) the percentage contribution of each retinal layer to the full thickness of the retina.

For normal control animals, the mean total thickness of the retina was 252.9 μm (SD 55.5), with a mean error of 8.8 μm. In addition, the mean PRL thickness was 75.5 μm (SD 26.2), and the mean ONL thickness was 61.8 μm (SD 19.2). The ONL consisted of 11.3 (SD 3.2) neural-cell rows, with a mean error of 0.5. The ORL thickness was 11.6 μm (SD 4.5). The mean INL thickness was 33.1 μm (SD 11.0), and the number of neural cells was substantially smaller in the INL than in the ONL. The INL consisted of 4.6 (SD 1.6) neural-cell rows, with a mean error of 0.3. The IRL was 4.1 times thicker than the ORL (47.2 μm (SD 17.5) versus 11.6 μm (SD 3.2)), and the mean thickness of the GCL plus NFL was 23.7 μm (SD 9.1)).

For animals with diabetes duration of 3 months, the mean total thickness of the retina was 262.1 μm (SD 39.3), with a mean error of 8.5 μm. In addition, the mean PRL thickness was 74.0 μm (SD 18.8), and the mean ONL thickness was 66.1 μm (SD 20.2). The ONL consisted of 11.7 (SD 2.9) neural-cell rows, with a mean error of 0.6. The ORL thickness was 12.4 μm (SD 3.1). The mean INL thickness was 33.6 μm (SD 8.8), and, similar to normal animals, the number of neural cells was substantially smaller in the INL than in the ONL. The INL consisted of 4.7 (SD 0.8) neural-cell rows, with a mean error of 0.2. The IRL was 3.9 times thicker than the ORL (47.9 μm (SD 11.7) versus 12.4 μm (SD 3.1)) and was somewhat thinner than in normal animals. The mean thickness of the GCL plus NFL was 28.1 μm (SD 12.6)). There was no significant difference in any of the examined parameters of retinal layers between rats with diabetes duration of 3 months and normal rats (Table 1). This was indicated also by the comparison of microphotographs of diabetic rats (Figs. 2-5) and normal rats (Fig. 1).

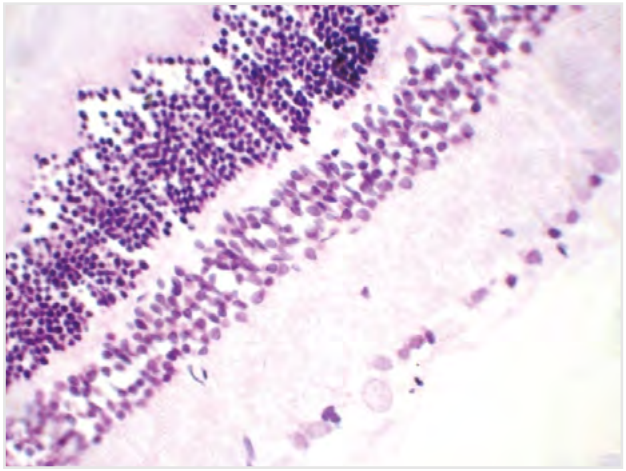

Fig. 5. Neural retina of a Wistar rat with 3-month diabetes duration. Note a neuronal cell undergoing lysis in the ganglion cell layer (arrow). Hematoxylin and eosin staining. Objective magnification, x40; eyepiece magnification, x7.

Findings on the percentage contribution of each retinal layer to the full thickness of the retina were also interesting. In Wistar rats, the GCL plus NFL was the thickest retinal layer (Table 1, Fig. 6), with a contribution of almost half of the total retinal thickness, both for normal and diabetic rats. In addition, the ORL was the thinnest retinal layer, with a contribution of less than 5% of the total retinal thickness, both for normal and diabetic rats.

Table 1. Comparison between diabetic Wistar rats and healthy controls in terms of thicknesses of separate retinal layers and the percentage contribution of individual retinal layers to the full thickness of the retina

Retinal layers	Retinal layer thickness (μm) mean ± error of mean (percentage contribution to the full thickness of the retina)		P-value
	Control group, n = 3	Diabetic animals, n=5	
Photoreceptor layer	75.5 ± 4.14 (29.85 %)	74.0 ± 3.85 (28.23%)	>0.05
Outer nuclear layer	61.8 ± 3.04 (24.44%)	66.1 ± 4.12 (25.22%)	>0.05
Outer retinal layer	11.6 ± 0.72 (4.59%)	12.4 ± 0.64 (4.73%)	>0.05
Inner nuclear layer	33.1 ± 1.74 (13.01%)	33.6 ± 1.75 (12.82%)	>0.05
Inner retinal layer	47.2 ± 2.77 (18.66%)	47.9 ± 2.39 (18.27%)	>0.05
Ganglion cell layer plus nerve fiber layer	23.7 ± 1.44 (9.37%)	28.1 ± 2.57 (11.11%)	>0.05

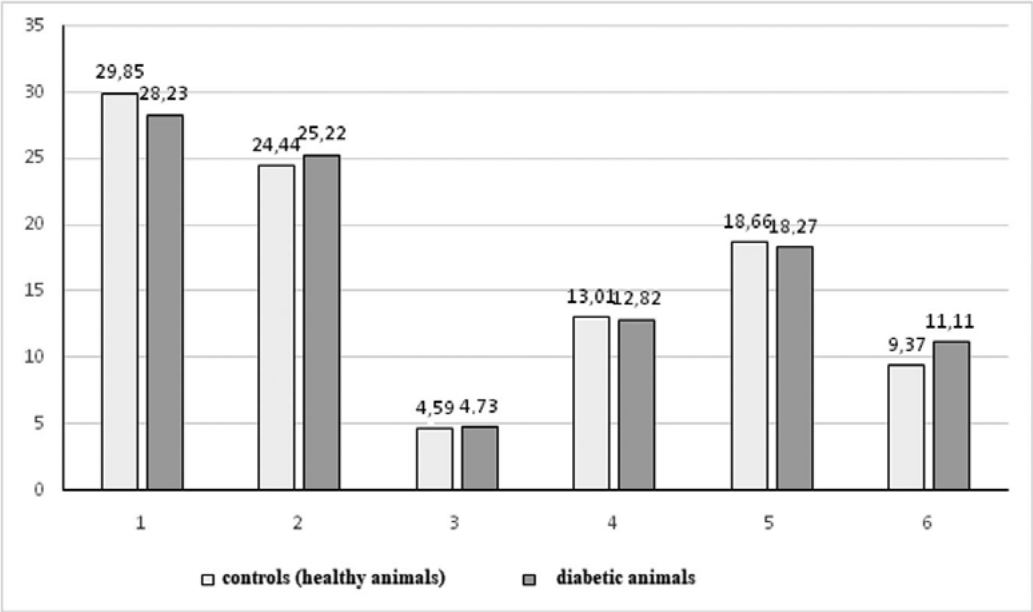

Fig. 6. Percentage contributions of individual retinal layers to the full thickness of the retina. Note: 1, photoreceptor layer; 2, outer nuclear layer; 3, outer retinal layer; 4, inner nuclear layer; 5, inner retinal layer; 6, ganglion cell layer plus nerve giber layer

Discussion

Unfortunately, the prevalence of DM in Ukraine is high, similar to other countries, with the number of people living with DM in the country as large as 2.3 million for 2021 [14], and with a contribution of 95% from type 2 DM [15]. Therefore, DM and its major complication, DR, present a challenge to Ukrainian ophthalmologists.

Our classical microscopic evaluation of histological specimens at the beginning of this study already allowed for making a preliminary conclusion on the absence of neurodegenerative changes in retinal layers in Wistar rats with STZ-induced diabetes (Figs. 1-5). This microscopic evidence was confirmed by morphometric calculations of the thicknesses of retinal layers and numbers of neural-cell rows in the INL and ONL of the retina. The point is that, thicknesses of these layers would decrease with a neurodegeneration-induced neural cell death and decrease in the number of neural cells in retinal layers. Therefore, the use of laborious morphometric calculations was well founded.

The results of our morphometric analysis gave us ground to state that there was no statistically significant difference in thicknesses of retinal layers or numbers of neural-cell rows in the INL and ONL of the retina between rats with diabetes duration of 3 months and normal rats. Therefore, this was evidence of the absence of retinal neurodegenerative changes in Wistar rats with STZ-induced diabetes duration. Because undoubtedly, if there was neurodegeneration of retinal neural tissue, not only the numbers of neural cells and neural-cell rows in both nuclear layers, but also the thicknesses of these layers would decrease. But actually there was no neurodegeneration of retinal neural tissue, which can be seen also in Fig. 6. To explain this, we should consider the retinal vascular bed of the rat. It is well known that, in rats, the retina, similar to that in humans, is well vascularized, which facilitates retinal blood supply. Such a vessel can be seen in Fig. 4.

A recent study by Ziablitsev and Vodianyk [16] assessed changes in retinal structure in Wistar rats 28 days after a single 50 mg/kg intraperitoneal STZ injection. Edema of all retinal layers, low-density nuclear layer neurons, hyperemic and dilated venules in the outer plexiform layer, loose nerve fibers, marked extracellular edema in the GCL and high susceptibility of the inner retinal layers to apoptosis were observed. In the current study, at three months after STZ injection, we, however, did not see such profound changes as those mentioned by Ziablitsev and Vodianyk [16]. We believe that this can be explained by the regeneration of beta cells of the rat's pancreas leading to spontaneous diabetes remission, which was found by Kern and Engerman as early as 1994 [17]. Alder and colleagues [18] provided much more evidence of the regeneration of beta cells in STZ rats. Hyperglycemia (> 22 mmol/L) was sustained for the first 36 weeks, following which blood glucose levels gradually and spontaneously recovered to normoglycemia by 90 weeks. The diabetic rats remained normoglycemic for the remaining 30 weeks of the experiment, which brought them close to the end of their natural life span [18].

Therefore, the diabetic model described in the current study hardly may be considered as the most convenient for examining neurogenerative changes in the retina with simple and readily available histological examination techniques. It should be taken into account that, it has been believed since the early 2000s that DR initially affects not the retinal vasculature but neural retinal tissue [19, 20] that undergoes neurodegeneration. Consequently, it could be considered that the Wistar rat is not the best species for studies on the phenomenon of neurodegeneration. Studies on morphometric parameters of retinal layers in other species (mice and rabbits) will allow identifying the species with the most apparent signs of retinal neurodegeneration in DM, and it is on these species that it would be possible to test potential medications for the treatment of DR.

References

1. Teo ZL, Tham YC, Yu M, Chee, et al. Global prevalence of diabetic retinopathy and projection of burden through 2045: systematic review and meta-analysis. Ophthalmology. 2021;128(11):1580-91. doi: 10.1016/j.ophtha.2021.04.027.
2. Hariprasad S, Holecamp N, MacCumber M, et al. Evidence-based management of diabetic eye diseases. Retinal Physician. 2017 April: 4-12.
3. Xu Y, Jiang Z, Huang J, et al. The association between toll-like receptor 4 polymorphisms and diabetic retinopathy in Chinese patients with type 2 diabetes. Br J Ophthalmol. 2015 Sep;99(9):1301-5. doi: 10.1136/bjophthalmol-2015-306677.
4. Giyasova AO, Yangieva NR. Comparing the effectiveness of brolucizumab therapy alone versus that combined with subthreshold micropulse laser expo-sure in the treatment of diabetic macular edema. J Ophthalmol (Ukraine). 2023; 2:16–20. doi: 10.31288/oftalmolzh202321620.
5. Malachkova NV, Kyryliuk ML, Komarovska IV. [Blood pressure in patients with diabetic retinopathy, type 2 diabetes mellitus and obesity]. Arkhiv oftalmologii Ukrainy. 2017;5(1):32-37. Russian.
6. Serdiuk VN, Kyryliuk ML, Ishchenko VA. Mathematical substantiation of the method for assessing the risk of progression of diabetic retinopathy with serum leptin determination in patients with metabolic syndrome and diabetes mellitus. J Ophthalmol (Ukraine). 2018;(2):15–22. doi: 10.31288/oftalmolzh/2018/2/1721.
7. Albini T, Regillo C, Drenser K, et al. Evidence-based management of diabetic eye diseases. Retinal physician. 2016; March: 4-14.
8. Barth T, Helbig H. [Diabetic retinopathy]. Klin Monatsbl Augenheilkd. 2021 Oct;238(10):1143-1159. doi: 10.1055/a-1545-9927.
9. Alifanov IS, Sakovych V.M. Prognostic risk factors for diabetic retinopathy in patients with type 2 diabetes mellitus. J Ophthalmol (Ukraine). 2022;(6):19–23. doi: 10.31288/oftalmolzh202261923.
10. Cho H, Alwassia AA, Regiatieri CV, et al. Retinal neovascularization secondary to proliferative diabetic retinopathy characterized by spectral domain optical coherence tomography. Retina. 2013;33:542–7. doi: 10.1097/IAE.0b013e3182753b6f.
11. Wykoff CC, YuHJ, Avery RL, et al. Retinal non-perfusion in diabetic retinopathy. Eye (Lond). 2022 Feb;36(2):249-56. doi: 10.1038/s41433-021-01649-0.
12. Walker J, Rykov SA, Suk SA, et al. [Diabetic retinopathy: complexity made simple: a monograph]. Kyiv: Biznes-Logika; 2013. Russian.
13. Maltsev EV, Zborovska AV, Dorokhova AE. [Fundamental aspects of the development and treatment of diabetic retinopathy]. Odesa: Astroprint; 2018. Russian.
14. Nevska AO, Pogosian OA, Goncharuk KO, et al. Detecting diabetic retinopathy using an artificial intelligence-based software platform: a pilot study. J Ophthalmol (Ukraine). 2024;1:27-32. doi: 10.31288/oftalmolzh202412731.
15. Rykov SO, Galytska IeP, Zhmuryk DV, Dufynets VA. Association of TLR4 rs1927911 polymorphism with diabetic retinopathy and diabetic macular edema in type 2 diabetic patients. J Ophthalmol (Ukraine). 2024;1(516):20-6. doi: 10.31288/oftalmolzh202412026.
16. Ziablitsev SV, Vodianyk VV. Retinal apoptosis and the effect of tyrosine kinase inhibition in experimental diabetes. J Ophthalmol (Ukraine). 2023;5:34-40. doi: 10.31288/oftalmolzh202353440.
17. Kern TS, Engerman RL. Comparison of retinal lesions in alloxan-diabetic rats and galactose-fed rats. Curr Eye Res. 1994; 13: 863-867.

18. Alder VA, Su EN, Yu DY, Cringle S, Yu P . Overview of studies on metabolic and vascular regulatory changes in early diabetic retinopathy. Austr NZ J. Ophthalmol. 1998;26(2):141-8.
19. Lieth E, Gardner TV, Barber AJ, et al. Retinal neurodegeneration: early pathology in diabetes. Clin Exp Ophthalmol. 2000 Feb;28(1):3-8. doi: 10.1046/j.1442-9071.2000.00222.x.
20. Barber AJ. A new view of diabetic retinopathy: a neurodegenerative disease of the eye. Prog Neuropsychopharmacol Biol Psychiatry. 2003;27:283-90.

Disclosures

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Author Contributions: *All authors meet the authorship criteria and contributed substantially to conception and design or acquisition of data, or analysis and interpretation of data, drafted or revised the version to be published. The authors are responsible for the content of the article. All authors state that they have not submitted and will not submit a similar paper for publication elsewhere in any language.*

Disclaimer: *The opinions presented in this article are those of the authors and do not necessarily represent those of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine", Odesa (Ukraine).*

Subjects of the study: *Animal experiments were performed in compliance with the Law of Ukraine on Protection of Animals from Cruel Treatment No. 3447-IV and European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes from the European Treaty Series (Strasbourg, 1986). The study was approved by the local Bioethics Committee of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine" (meeting minutes dated October September 14, 2024).*

Data Availability Statement: *All the data obtained or analyzed during this study are reported in the article.*

Abbreviations: *DR, diabetic retinopathy; DM, diabetic mellitus*