Morphometric analysis of retinal structural components in rabbits in experimental diabetes mellitus

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Морфометричний аналіз стану структурних елементів сітчастої оболонки очей кролика при експериментальному цукровому діабеті

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Abstract

Purpose. *To perform morphometric analysis of retinal structural components in rabbits in experimental diabetes mellitus (DM) in an attempt to digitize possible retinal neurodegenerative changes foe comparison with other species with induced DM.*

Material and Methods*: Stained hematoxylin and eosin histological sections from 19 eyes of 19 Chinchilla rabbits were retrospectively reviewed. Of the 19 eyes, 11 were from 11 rabbits at 16–17-week diabetes duration, and 4 were from 4 healthy rabbits. 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 plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), and ganglion cell layer (GCL)/ nerve fiber layer (NFL). The numbers of nuclear rows in both nuclear layers were calculated visually. An eyepiece micrometer was used to perform retinal layer thickness measurements.*

Results*. Total retinal thickness (mean ± standard error of mean) was 110.6 ± 3.77 µm for healthy animals versus 111.0 ± 2.33 µm for diabetic animals; PRL thickness, 27.8 ± 2.11 µm versus 21.9 ± 1.30 µm; ONL thickness, 28.4 ± 0.97 µm versus 11.0 ±0.60 µm; OPL thickness, 8.1 ± 1.37 µm versus 27.8 ± 0.85 µm; INL thickness, 13.8 ± 0.99 µm versus 6.1 ± 0.61 µm; OPL thickness, 16.0 ± 1.05 µm versus 18.3 ± 0.65 µm; and GCL/NFL thickness, 16.5 ± 1.04 µm versus 25.8 ± 0.64 µm, respectively. The number of nuclear rows in the ONL was 6.94 ± 0.17 for healthy animals versus 1.96 ± 0.10 for diabetic animals. The number of nuclear rows in the INL was 3.03 ± 0.09 for healthy animals versus 1.57 ± 0.05 for diabetic animals. Obviously, the retina of diabetic rabbits showed pronounced signs of neurodegeneration.*

Conclusion. *Microscopic pictures of retinal sections of and the estimated thicknesses of individual retinal layers and numbers of nuclear rows in the ONL and INL from, diabetic rabbits at 16–17-week diabetes duration indicate that the dithizone-induced diabetes rabbit model used may be considered suitable for histological (hematoxylin and eosin) evaluation of retinal degenerative changes.*

Keywords: *choroid, choriocapillaries, retina, photoreceptor cells, optic nerve, chronic alcohol intoxication, ultrastructure.*

Резюме

Мета. *провести морфометричний аналіз стану структурних елементів сітківки очей кролика при експериментальному цукровому діабеті (ЦД) для отримання цифрових показників можливих нейродегенеративних змін цієї оболонки ока, необхідних для порівняння з сітківками тварин інших видів з цією же хворобою.*

Матеріал і методи. *На архівних гістологічних зрізах, пофарбованих гематоксилін-еозином, очей кроликів шиншила, хворих цукровим діабетом (ЦД) до 16–17 тижнів (11 кроликів, 11 очей) та здорових тварин (4 кролика, 4*

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ока), вимірювалася загальна товщина сітківки в мікрометрах (мкм), потім товщина таких її шарів, як шар фоторецепторів, зовнішній ядерний, зовнішній сітчастий, внутрішній ядерний, внутрішній сітчастий, гангліозних клітин та нервових волокон. Підраховували візуально кількість рядів нейронів в обох ядерних шарах. Шари сітківки вимірювалися за допомогою окуляр-мікрометра.

Результати. *Товщина (середнє значення з його помилкою) сітківки ока здорового контрольного кролика складає 110,6 ± 3,77 мкм, а хворого ЦД 111,0 ± 2,33 мкм. Товщина окремих її шарів становить: шару фоторецепторів здорового кролика – 27,8±2,11 мкм, а при ЦД – 21,9±1,30 мкм; зовнішнього ядерного шару у здорового кролика – 28,4 ±0,97 мкм, а при ЦД – 11,0 ±0,60 мкм; зовнішнього сітчастого шару у здорового кролика – 8,1 ±1,37 мкм, а при ЦД – 27,8±0,85 мкм; внутрішнього ядерного шару у здорового кролика – 13,8±0,99 мкм, а при ЦД – 6,1±0,61 мкм; внутрішнього сітчастого шару у здорового кролика – 16,0±1,05 мкм, а при ЦД – 18,3±0,65 мкм; гангліозних клітин та нервових волокон у здорового кролика – 16,5 ±1,04 мкм, а при ЦД – 25,8 ±0,64 мкм. Кількість рядів нейронів у зовнішньому ядерному шарі у здорового кролика 6,94±0,17, а при ЦД 1,96±0,10. Кількість рядів нейронів у внутрішньому ядерному шарі у здорового кролика 3,03±0,09, а при ЦД 1,57±0,05. Очевидно, що в сітківці кролика мають місце виражені ознаки нейродегенерації.*

Висновок. *Мікроскопічна картина сітківки ока кролика з цукровим діабетом протягом 16–17 тижнів та кількісні підрахунки товщини її окремих шарів, а також кількості рядів нейронів в ядерних шарах свідчать, що використана модель цукрового діабету може рахуватися як зручна для гістологічного вивчення нейродегенеративних змін цієї оболонки з використанням оглядових методів фарбування препаратів (гематоксилін-еозин).*

Ключові слова: *діабетична ретинопатія, цукровий діабет, сітківка, морфометрія, нейродегенерація.*

Introduction

Diabetes mellitus (DM) is a key public health problem and one of the most common diseases worldwide, associated with premature death and disability. The global burden of diabetes has markedly increased in recent decades, and the disease has attained the status of a global pandemic. It has been estimated that in 2024, some 588.7 million people worldwide had diabetes. If the trends continue, by 2050, 852.5 million of people aged 20-79 years will have diabetes. This will result in an increase in the number of people with diabetic retinopathy (DR) since DR affects over one-third of all people with diabetes [1, 2, 3, 4].

DR may appear in type 1 diabetes patients in the first 3-5 years of diabetes [5]. Among individuals with diabetes, global prevalence was 22.27% for DR, 6.17% for vision-threatening DR (VTDR), and 4.07% for clinically significant macular edema (CSME). In 2020, the number of adults worldwide with DR, VTDR, and CSME was estimated to be 103.12 million, 28.54 million, and 18.83 million, respectively; by 2045, the numbers are projected to increase to 160.50 million, 44.82 million, and 28.61 million, respectively [6].

The risk of DR is increased in the presence of diabetic nephropathy and lower limb angiopathy [4]. Additionally, one should keep in mind that blindness is much more common in diabetics than in general population. The development of proliferative DR with vitreoretinal neovascularization can result in vitreous hemorrhage, neovascular glaucoma and tractional retinal detachment, leading to potential blindness [7]. Diabetic macular edema (DME) is another relatively late diabetic complication leading to potential blindness [8]. Because “diabetes is still a major cause of blindness in adults older than 65 years of age”, DR is a challenge for ophthalmologists [9]. Monographs addressing the international knowledge on DR and its management have been published in Ukraine [9, 10].

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, and with a contribution of 95% from type 2 DM. Artificial intelligence (AI) has been already used to detect DR in diabetics [11], and an association of TLR4 rs1927911 with DR has been reported [12].

DR has been classically considered to be a retinal microvascular complication of diabetes [13, 14]. Fundamental and clinical studies, however, provide growing evidence to suggest that retinal neurodegeneration is an early event in the pathogenesis of DR which precedes visible vascular changes characteristic of the disease [15, 16].

Neurodegenerative process in DM is characterized by early retinal cell death and glial changes such as increased reactivity and dysfunction of microglia, astrocytes and Müller cells [17, 18, 19]. The clinical significance of neurodegenerative changes is that they are strongly associated with the abnormalities in visual function (reduced contrast sensitivity, impaired color sensation and changes in the electroretinogram) which develop before the onset of DR [20, 21, 22].

Methods for the treatment of DR are developed through animal research. Morphometric studies of retinal structures in various species of diabetic laboratory animals allow identifying the species exhibiting the most profound neurodegenerative changes, which justifies the rationale of using them in experimental studies and the development of the methods for the prevention and treatment of DR. This may become a key strategic solution for preventing irreversible vision loss in the preclinical stages of the disease.

We have previously reported on morphometric studies on the status of major retinal neural components in rats and mice [23, 24].

The purpose of this study was to perform morphometric analysis of retinal structural components in rabbits in experimental DM in an attempt to digitize possible retinal neurodegenerative changes for comparison with other species with induced DM.

Material and Methods

Preparations from 19 eyes of 19 Chinchilla rabbits from 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 the 19 eyes, 11 were from 11 diabetic rabbits at 16–17-week diabetes duration, and 4 were from 4 healthy rabbits. Diabetes was induced by an intravenous dose of 35 mg/kg dithizone. Five-µm stained hematoxylin and eosin histological sections were examined with a Laboval 4 microscope (Karl Zeiss, Jena, Germany) with a Canon PowerShot A480 camera attachment. An eyepiece micrometer was used to perform retinal layer thickness measurements.

These preparations have never been used previously for morphometric calculations. To perform morphometric calculations, we employed a 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 plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), and ganglion cell layer (GCL)/ nerve fiber layer (NFL). The numbers of nuclear rows in the INL and ONL were calculated visually.

JASP software was used for the analysis of measurement data. Mean and standard deviation values were calculated for each sample. The significance of differences in retinal characteristics examined between the eyes of diabetic animals and the eyes of healthy animals was also assessed. 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 total thickness of the retina was calculated and compared among the two groups.

Results

The structure of a normal rabit retina has the following major features. First, a normal rabbit retina (but not that of some other species) contains two bands of myelinated nerve fibers, the medullary rays, extending nasally and temporally from the optic disc and having blood vessels of their own. Second, the rabbit retina has no macula but a horizontal band, known as the "visual streak", which is located beneath the optic nerve. Finally, the retinal pigment epithelium (RPE) in the Chinchilla rabbit eye contains melanin granules.

Fig. 1 shows the normal rabbit retina.

The microscopic structure of the diabetic rabbit retina is different from normal in that it exhibits not only normally structured regions, but also simultaneously: (a) atrophic fragments with abruptly decreased either total retinal thickness or the thickness of one retinal layer; (b) local thickening of one retinal layer; and (c) various cell types with pathological changes. All we can do is try once more to confirm microscopically the presence of well-known features of structural retinal remodeling which develop due to the changes caused by DR.

In a dithizone-induced rabbit model of diabetes, the retina exhibits marked neurodegeneration. This was evidenced by numerous findings found while reviewing histological sections of the eyes of experimental animals during their morphometric measurements. The major of these findings were as follows:

- Alterations in normally layer-by-layer structure of the retina
- A wavy surface of the inner retina
- Atrophic retinal changes including local complete loss of the PRL
- Frequent loss or RPE cells
- Displacement of nuclei from the ONL to the PRL
- Nuclei from the ONL and INL were intermingled due to a local complete loss of the OPL
- Lysis and vacuolization of numerous GCL cells
- Fragments of neurons that underwent degeneration in the ONL and INL
- Areas of retinal gliosis

Of special interest was the presence of retinal areas that were structurally normal or close to normal. Given the above changes in the diabetic rabbit retina which can be easily viewed with conventional microscopy, it is understood that morphometric measurements should demonstrate changes in the thicknesses of various retinal layers and numbers of nuclear rows in retinal nuclear layers.

Figures 2-5 show micrographs of retinal sections from the diabetic rabbits (16-17-week diabetes duration). Numerous quantitative and qualitative alterations in the retinal structure from the eyes from the diabetic rabbits (16-17-week diabetes duration) can be seen (Figures 2-5).

Interestingly, the fragments seen in Figs 2 and 3 are easily recognizable as those of the retina due to a well-organized multilayered structure, whereas the relevant path-

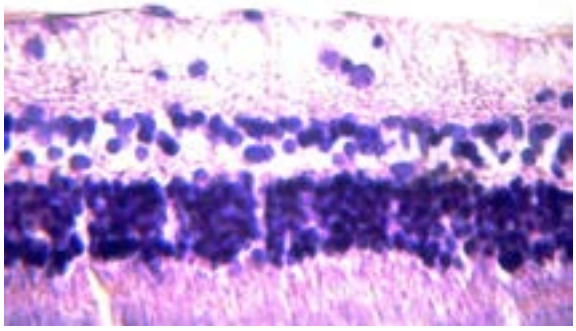

Fig. 1. Retina of a healthy control rabbit. The outer nuclear layer contains as much as 7-8 rows (compared with 2-3 rows in the inner nuclear layer) and is twice as thick as the inner nuclear layer. The outer plexiform layer is much thinner than the inner plexiform layer. Hematoxylin and eosin staining. Objective magnification, x40; eyepiece magnification, x16.

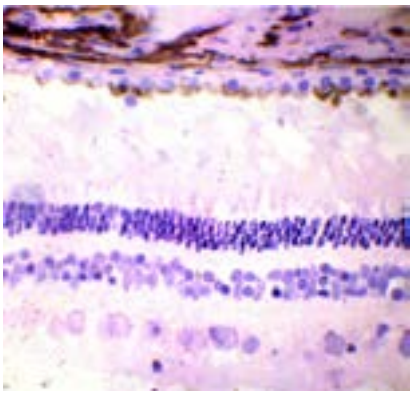

Fig. 2. The retina and choroid of a rabbit with 16-17-week diabetes duration. Although retinal structure appears normal, the outer nuclear layer contains only 3-4 rows and is as thick as the inner nuclear layer. Hematoxylin and eosin staining. Objective magnification, x40; eyepiece magnification, x7.

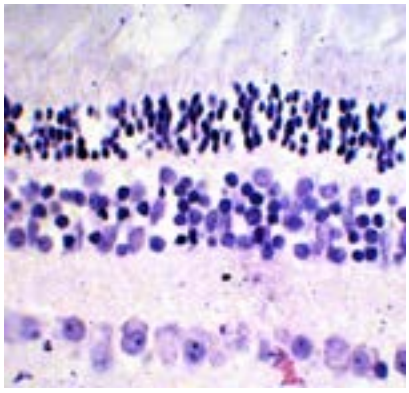

Fig. 3. The retina (in the visual streak region) of a rabbit with 16-17-week diabetes duration. Although retinal structure appears normal, the outer nuclear layer contains only 4-6 rows and is thinner than the inner nuclear layer. Hematoxylin and eosin staining. Objective magnification, x40; eyepiece magnification, x7.

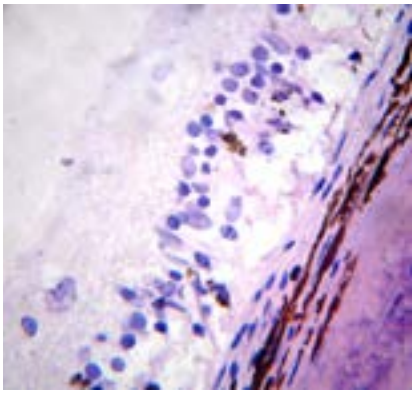

Fig. 4. The retina (in the visual streak region) of a rabbit with 16-17-week diabetes duration. Note edematous cavities at the location of outer retinal layers. Two to four nuclear rows are seen at the former location of both retinal nuclear layers and appear separated by the outer plexiform layer. Hematoxylin and eosin staining. Objective magnification, x40; eyepiece magnification, x16.

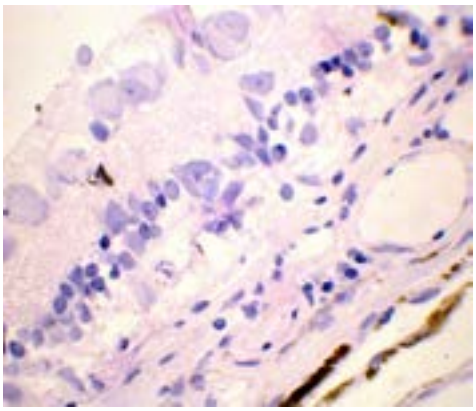

Fig. 5. The retina (in the visual streak region) of a rabbit with 16-17-week diabetes duration is seen fused with the choroid. Nuclei of the two retinal nuclear layers appear intermingled with each other, and only 2-3 nuclear rows are seen. No photoreceptor layer, pigment epithelial layer or outer plexiform layer is seen. The inner plexiform layer appears somewhat thinned. Hematoxylin and eosin staining. Objective magnification, x40; eyepiece magnification, x7.

ological changes are quantitative in nature and are specified in figure legends. The tissue fragments seen in Fig. 4 and especially Fig 5, however, exhibit a structure which is substantially different from that of a normal rabbit retina. Thus, the tissue fragments seen in Fig. 4 exhibit only 2–3 nuclear rows at their original locations in the ONL and INL, whereas Fig. 5 exhibits ganglion cells of remnants of the visual streak.

Table 1 and Fig. 6 compare healthy and diabetic rabbits with regard to morphometrics of the retinal layers.

In healthy rabbits, total retinal thickness was 110.6 ± 3.77 μm (95% CI, 103.06–118.14 μm), PRL thickness, 27.8 ± 2.11 μm (95% CI, 23.6–31.9 μm), and ONL thickness, 28.4 ± 0.97 μm (95% CI, 26.5–30.3), and, the number of nuclear rows in the ONL was 6.94 ± 0.17 (95% CI, 6.61–7.27). Additionally, OPL thickness was 8.1 ± 1.37 μm (95% CI, 5.4–10.8 μm), and INL thickness, 13.8 ± 0.99 μm (95% CI, 11.9–15.7 μm), and, the number of nuclear rows in the INL was 3.03 ± 0.09 (95% CI, 2.86–3.20) which was less than in the ONL. Moreover, the IPL was thicker than the OPL, and its thickness was 16.0 ± 1.05 μm (95% CI, 13.9–18.1 μm), whereas the thickness of the GCL and NFL was 16.5 ± 1.04 μm (95% CI, 14.5–18.6 μm). It is well understood that it is hardly reasonable to compare the above retinal measurements in normal rabbit eyes and those in diabetic rabbit eyes (16-17-week diabetes duration), because the latter eyes already exhibit the qualitative structural changes in the retina. However, this data can be useful for comparison with other mammal species.

In diabetic rabbits (16-17-week diabetes duration), total retinal thickness was 111.0 ± 2.33 μm (95% CI, 106.34–115.66 μm), PRL thickness, 21.9 ± 1.30 μm (95% CI, 19.4–24.5 μm), and ONL thickness, 11.1 ± 0.60 μm (95% CI, 9.9–12.3), and, the number of nuclear rows in the ONL was 1.96 ± 0.10 (95% CI, 1.75–2.16). Additionally, OPL thickness was 27.8 ± 0.85 μm (95% CI, 26.1–29.5 μm). This was due to the fact that, at many retinal locations, the ONL merged with the INL, the displacement of nuclei from the ONL and INL to the OPL occurred, and thus the OPL became wider than the IPL. INL thickness was 6.1 ± 0.61 μm (95% CI, 4.9–7.3 μm), and, the number of nuclear rows in the INL was 1.57 ± 0.05 (95% CI, 1.46–1.68) which was less than in the ONL. The IPL was narrower than the OPL, and IPL thickness was 18.3 ± 0.65 μm (95% CI, 17.0–19.5

Table 1. Comparing thicknesses of individual retinal layers between healthy rabbits and diabetic rabbits with a diabetes duration of 16-17 weeks

Retinal layer	Retinal layer thickness (μm), mean ± standard error of mean		95% CI, healthy control animals	95% CI, diabetic animals
	Healthy control animals, n = 4	Diabetic animals, n = 11		
Photoreceptor layer	27.8 ± 2.11	21.9 ± 1.30*	23.6 - 31.9	19.4 - 24.5
Outer nuclear layer	28.4 ± 0.97	11.1 ± 0.60*	26.5 - 30.3	9.9 - 12.3
Outer plexiform layer	8.1 ± 1.37	27.8 ± 0.85*	5.4 - 10.8	26.1 - 29.5
Inner nuclear layer	13.8 ± 0.99	6.1 ± 0.61*	11.9 - 15.7	4.9 - 7.3
Inner plexiform layer	16.0 ± 1.05	18.3 ± 0.65	13.9 - 18.1	17.0 - 19.6
Ganglion cell layer plus nerve fiber layer	16.5 ± 1.04	25.8 ± 0.64*	14.5 - 18.6	24.6 - 27.1

Note: *, statistically significant difference between diabetic and control animals; n, number of rabbits (eyes); CI, confidence interval

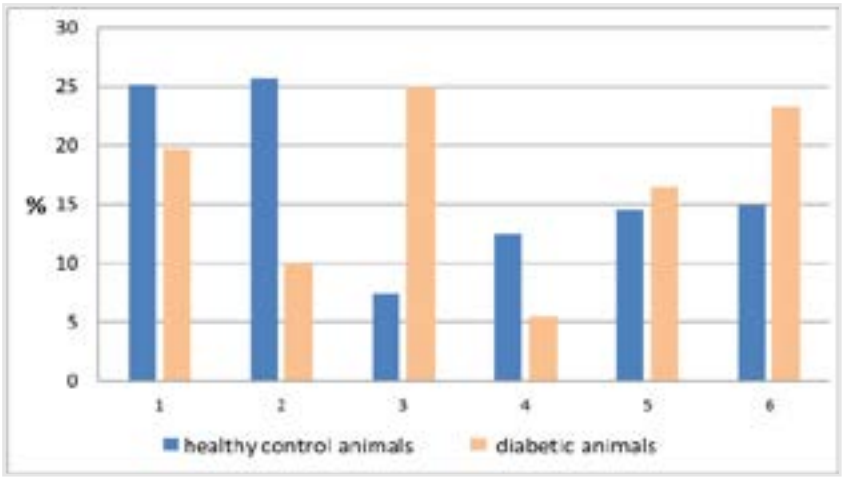

Fig. 6. Percentage contributions of thicknesses of individual retinal layers to the total thickness of the retina.

Note: 1, photoreceptor layer; 2, outer nuclear layer; 3, outer plexiform layer; 4, inner nuclear layer; 5, inner plexiform layer; 6, combined ganglion cell layer and nerve fiber layer

μm). It is noteworthy that there was no significant difference ($p > 0.05$) in the thickness of the IPL (but not the thickness of any other retinal layer or the number of nuclear rows) between eyes of diabetic rabbits and eyes of normal rabbits. The combined thickness of GCL and NFL was 25.8 ± 0.64 μm (95% CI, 24.6–27.1 μm).

Findings on the percentage contribution of each retinal layer to the total thickness of the retina (Fig. 6) will be also interesting doe comparison with other species. PRL and ONL contributions to total retinal thickness were 25.14% and 25.68%, respectively, for the retina of healthy rabbits versus 19.73% and 10.0%, respectively, for that of diabetic rabbits. This indicates that the ONL in diabetic animals was about 2.5-times thinner than in normal animals (11.1 ± 0.60 μm versus 28.4 ± 0.97 μm). The OPL contribution to total retinal thickness was about three-fold larger in diabetic animals than in normal animals (25.05% versus 7.32%) due to displacement of nuclei from the INL and ONL to the OPL. INL and IPL contributions to total retinal thickness were 12.48% and 14.47%, respectively, for the retina of healthy rabbits versus 5.49% and 16.49%, respectively, for that of diabetic rabbits. Moreover, the contribution of the combined thickness of GCL and NFL to total retinal thickness was 14.92% for the retina of healthy rabbits versus 23.24% for that of diabetic rabbits.

It is easily seen from Fig. 6 that it is diabetes in the rabbit model that is accompanied by marked retinal changes in the form of DR rather early after the onset of the disease. This phenomenon and its potential causes are discussed below.

Discussion

The results of this study demonstrate that diabetic rabbit eyes exhibited clear retinal structural changes indicating the development of DR. These changes particularly include the loss of a typical multilayer structure in some retinal areas, development of gliosis in other retinal areas, and reductions in the numbers of nuclear rows in retinal nuclear layers. Thickness changes in all retinal layers of diabetic rabbit eyes were profound and significant with the exception of the IPL. This feature makes our rabbit model of DR different from DR rodent models.

We have found no retinal morphometric abnormalities in diabetic rats [23] which are known to have a greater pancreatic better-cell regeneration rate than humans [25, 26]. Additionally, we have found significant changes in the thickness of internal retinal layers, but no microscopic evi-

dence of neurodegeneration in diabetic mice even at longer (as long as 6 months) [24] time points than in rabbits or rats. A significant vulnerability to retinal neurodegeneration in rabbits is likely to be explained by the features of retinal blood supply. The rodent inner retina (but not the rodent inner retina) has vessels that contribute to adequate retinal blood supply. The blood vessels (even large-caliber blood vessels) easily seen in retinal sections are located in the nasal and temporal medullary rays, and provide blood supply to these rays [27]. It is this feature of the retinal blood supply in the rabbit that makes the rabbit retina relatively vulnerable to stress exposure. The pathophysiological mechanisms of neurodegeneration involve deficits of neurotrophic factors [such as brain-derived neurotrophic factor (BDNF)], marked oxidative stress, excessive expression of the renin-angiotensin system, and glutamate metabolism abnormalities leading to glutamate excitotoxicity and neuromal death [28, 29, 30].

With regard to PRL and ONL thinning, it is well known that photoreceptor cell apoptosis has a significant role in retinal disease and aging [31].

Our findings are in agreement with the reports that retinal neurodegenerative changes preceded the development of DR, which was confirmed by optical coherent tomography (OCT) evidence of RNFL, IPL and GCL thinning [20, 32, 33].

Special attention has been given to the role of neuroinflammation in the development of DR [34, 35]. The fact that Müller glial cells are a major source of various factors, including inflammatory mediators, indicates that the activation of retinal glial cells may play an early role in inducing the inflammation responsible for retinal damage in late stages of the disease. Increased levels of the proinflammatory cytokines, interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor (TNF)-α and monocyte chemoattractant protein-1 (MCP-1), in patients with non-proliferative DR have been reported [36]. In the current study, the INL in diabetic rabbits was almost half as thick as in normal rabbits, and it is this layer that houses Müller cell nuclei. This supposes that, in early DR in rabbits, glial lesions develop with the development of neuroinflammation, contributing to retinal neurodegenerative changes. Possible neuroinflammation in the DR rabbit model warrants further research and may be promising in the context of the search for pharmacological correction of neuroinflammation in DR.

Therefore, microscopic pictures of retinal sections of and the estimated thicknesses of individual retinal layers and numbers of nuclear rows in the ONL and INL from, diabetic rabbits at 16–17-week diabetes duration indicate that the dithizone-induced diabetes rabbit model used may be considered suitable for histological (hematoxylin and eosin) evaluation of retinal degenerative changes.

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.

Disclaimers

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Conflict of Interest

The authors declare that they have no actual or potential conflict of interest that could inappropriately influence, or be perceived to influence, the subject matter or material discussed in this manuscript.

Study objects

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 September 14, 2024).

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations

DR, diabetic retinopathy; DM, diabetes mellitus, IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor α

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