ORIGINAL ARTICLE

CONTENTS

The second week corneal changes in rodents model of streptozotocin-induced diabetes

Oleksandr M. Slobodian[1], Zoriana Z. Masna[2], Yaroslav I. Penishkevych[1], Khrystyna I. Rudnytska[2], Ilona V. Chelpanova[2], Olena V. Smolkova[2], Kostyantyn I. Voytsenko[3]

[1]BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

[2]DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

[3]MUNICIPAL ENTERPRISE "CENTRAL CITY HOSPITAL OF CHERVONOGRAD CITY COUNCIL ", CHERVONOGRAD, UKRAINE

ABSTRACT

Aim: Installation the changes of the microstructural rearrangement of the layers of the rat cornea at the end of the second week of experimental streptozotocin-induced diabetes.

Materials and Methods: The research was conducted on 15 sexually mature, outbred white male rats, weighing 120-130 g. Two groups of animals were used in the work: the first group with developing diabetes (2 weeks after administration of streptozotocin); the second group served as control and received injections of 0.9% physiological solution for 2 weeks.

Results: Basal cells were located loosely, lost their columnar shape, basal cells of a rounded shape were visualized. The cytoplasm of individual basal cells was swollen and contained small acidophilic granules. At the same time, the cells of the basal layer with lighted vacuolated cytoplasm were visualized. Epitheliocytes of the middle layer of the outer epithelium of the cornea were chaotically located in individual areas, lumps of intensively condensed chromatin were often visualized in their nuclei. Focal destruction of the cells of the surface layer of the cornea and focal layering of fine-grained acidophilic masses in such areas were observed in places. The endothelium of the anterior chamber of the eye was preserved in most areas of the cornea.

Conclusions: At the end of the second week of experimental streptozotocin induced diabetes mellitus, we established pathomorphological manifestations indicating the initial phenomena of diabetic keratitis.

KEY WORDS: eye, membranes, cornea, experiment, diabetes, rat

Wiad Lek. 2024;77(9):1910-1915. doi: 10.36740/WLek/195141 DOI

INTRODUCTION

Diabetes mellitus is one of the most widespread endocrine diseases, which currently occupies one of the leading places in terms of medical and social importance, next to cardiovascular and oncological pathologies. Nowadays, the number of people with diabetes in Ukraine reaches 1.5-3% of the entire population [1]. Due to wide distribution of this pathology, the variety of complications, the severity of manifestations and the difficulty in selecting treatment, a thorough and comprehensive study of changes in the microstructural organization of tissues of the affected organs is indicated. Generalized vascular damage, specifically at the microcirculatory level is characteristic of both insulin-dependent and non-insulin-dependent types of diabetes, and largely determines the course and prognosis of the disease [2].

The incidence of ophthalmic diseases ranges globally approximately from 65% to 98%. Numerous clinical and experimental studies have established the relationship between eye tissue diseases and the state of somatic health, in particular, the state of the endocrine system and, as a result, the development of keratitis [3-8]. Diabetes mellitus significantly involves changes in tissues of the eyeball. The most numerous and at the same time contradictory information concerns the vascular concept of chronic damage to the membranes of the eyeball, which was formulated in the last century [9,10]. To date, it has not been definitively established whether the nerve fiber is primarily affected, as a result of a trophic disturbance, or a vessel, as the object of neurotrophic influence [11].

With a chronic, ongoing state of hyperglycemia, there is a higher probability of bacterial damage to the structures of the eyeball, in particular the cornea [12, 13]. On the one hand, this may be related to a change in the conjunctival flora in this group of patients due to an increase in the content of gram-positive cultures (mainly coagulase-negative

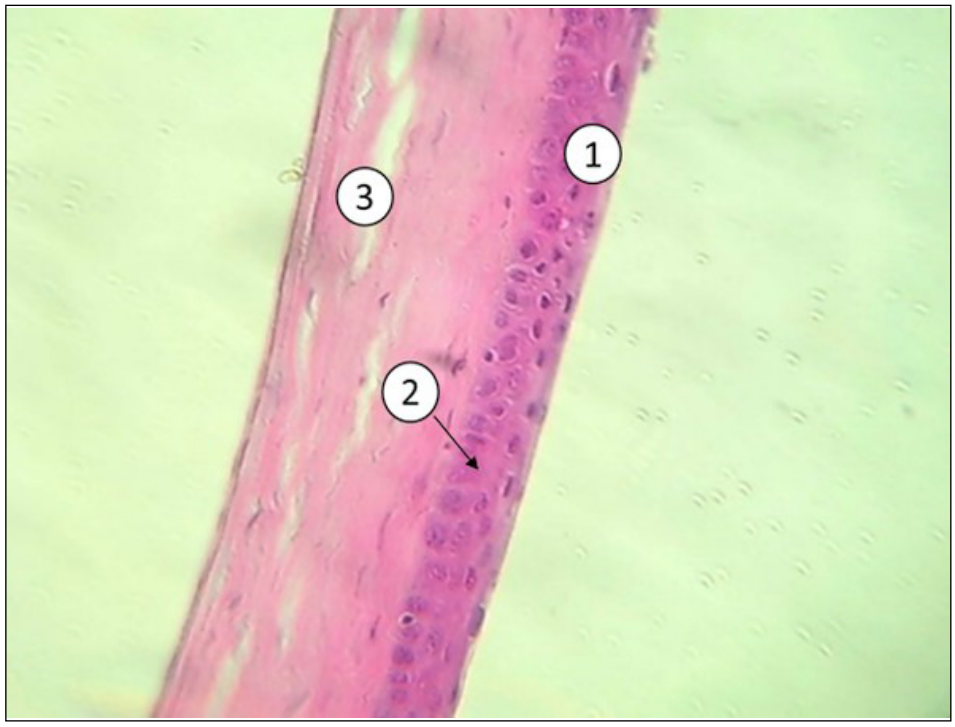

Fig. 1. Cornea at the end of the second week of experimental diabetes. Hematoxylin and eosin staining. Coll. x 400.
1-loose arrangement of cells of the basal layer of the outer epithelium of the cornea; 2-enlightenment of the cytoplasm of individual epitheliocytes of the basal layer; 3-delamination of the connective tissue plates of the main substance of the cornea.

staphylococcus) due to an increase in the concentration of glucose in tears and the frequent use of antibiotics [14].

On the other hand, specific structural and functional changes of the corneal tissues due to diabetes, is characterised by the development of diabetic keratopathy, which occurs as the background of diabetic corneal neuropathy. According to various authors, diabetic keratopathy occurs in 50–70% of patients with diabetes and is characterized by an asymptomatic course [12, 15].

However, eye trauma or any other surgical injury of a cornea being in such condition can lead to incomplete and delayed regeneration and is often the cause of reduced visual acuity due to clouding of the stroma and uneven surface [12, 15].

Bacterial keratitis occurs more often in patients with diabetes than in the general population, and has a more severe course [16–18]. A key role in the development and regulation of the inflammatory reaction is controled by cytokines — a group of mediators of intercellular interaction of a protein nature, which is the main non-specific humoral factor of immunity. According to their biological effect, cytokines are conditionally divided into pro-inflammatory and anti-inflammatory [19]. In bacterial keratitis, there is an increase in the expression of pro-inflammatory cytokines (interleukin 1α (IL-1 α) and interleukin 6 (IL-6)) in the tear fluid of the diseased eye [20, 21] and anti-inflammatory (interleukin 10 (IL-10)) — in tear fluid of the contralateral eye [20]. In patients with diabetes mellitus, the presence of chronic systemic inflammation of low intensity has been proven, which leads to an increase in the concentration of pro-inflammatory factors in blood plasma and biological fluids in the absence of an immunostimulator [22] and a change in the body's response to inflammatory diseases [23–26].

However, immunological aspects of bacterial keratitis in patients with diabetes have not been identified to date. Taking into account the above, diabetic damage to the structures of the eyeball will continue to be an actual problem of the possible development of diabetic damage to the cornea.

AIM

The aim was to find out the peculiarities of the microstructural rearrangement of the layers of the rat cornea at the end of the second week of experimental streptozotocin-induced diabetes.

MATERIALS AND METHODS

The research was conducted on 15 sexually mature, outbred white male rats, weighing 120-130 g. Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma, ltd) at the rate of 7 mg per 100 g of body weight (prepared in 0.1 mol citrate buffer, pH=4.5). The diabetes development was monitored during 2 weeks of blood glucose level increase, which was measured by the glucose oxidase method. The research was conducted from the second week of the experiment on animals with a glucose level of more than 13.48 mmol per 1 liter. Two groups of animals were used in the work: the first group (10 animals) with

Fig. 2. Cornea at the end of the second week of experimental streptozotocin diabetes. Hematoxylin and eosin staining. Coll. x 400.
1-acidophilic layers on the surface of the outer epithelium of the cornea.

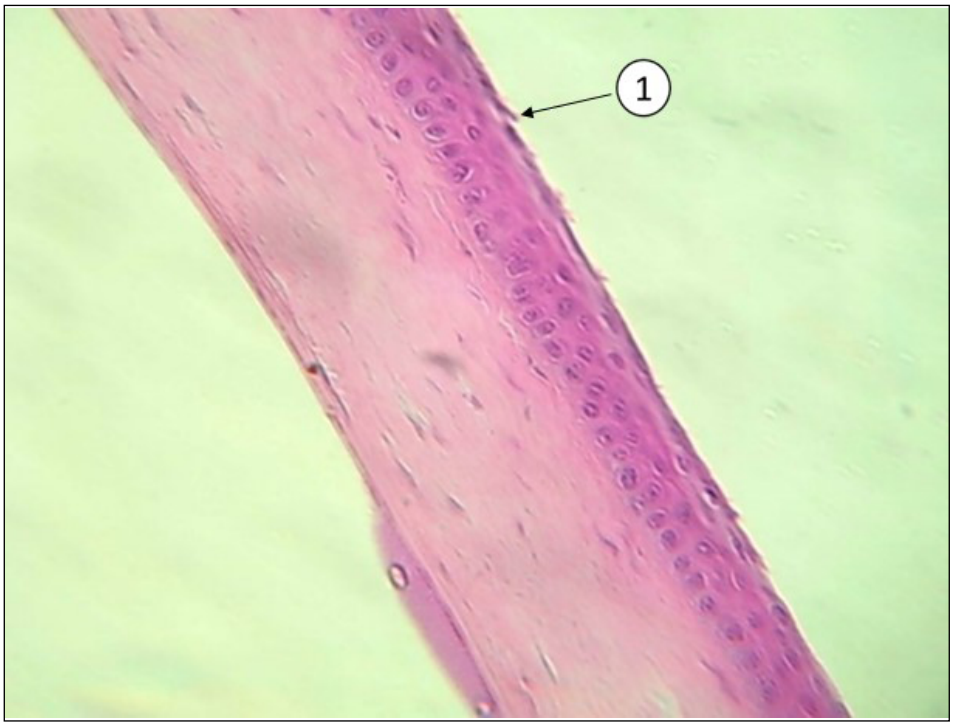

Fig. 3. Cornea at the end of the second week of experimental streptozotocin diabetes. Hematoxylin and eosin staining. Coll. x 400.
1- focal destruction of the surface layer of the outer epithelium of the cornea.

developing diabetes (2 weeks after administration of streptozotocin); the second group served as control (5 animals) and received injections of 0.9% physiological solution for 2 weeks.

All animals were kept in vivarium conditions and the procedures related to housing, care, labeling and all other manipulations were carried out in compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [Strasbourg, 1985], "General ethical principles of experiments on animals", adopted by the First National Congress on Bioethics [Kyiv, 2001], Law of Ukraine No. 3447 - IV "On the Protection of Animals from Cruelty". The Bioethics Commission of the Lviv National Medical University named after Danylo Halytsky found that the conducted scientific research meets the ethical requirements in accordance with the order of the Ministry of Health of Ukraine No.

231 of November 1, 2000 (protocol No. 10 of December 26, 2011), (protocol No. 2 of February 20, 2012 year). Before taking the material of the experimental site, the animal was removed from the experiment with the help of diethyl ether. The eyeballs of rats were used for microscopic examination (intersecting in the area of the limbus). Preparations for histological examination were prepared according to the generally accepted method [28]. Microscopic studies and photography of the preparations were carried out using an MBI-1 microscope and a Nicon D 3100 digital camera.

RESULTS

Microstructural examination of the cornea at the end of the second week of experimental streptozotocin-induced diabetes of the cornea of laboratory rats revealed that the outer epithelium of the cornea retained its integrity. In the epithelium, the basal, intermediate and surface layers were clearly visualized. In some areas of the cornea, the basal cells were located loosely, lost their columnar shape, and basal cells of a rounded shape were visualized (Fig. 1).

The cytoplasm of individual basal cells was swollen and contained small acidophilic granules. At the same time, the cells of the basal layer with lighted vacuolated cytoplasm were visualized.

Epitheliocytes of the middle layer of the outer epithelium of the cornea were chaotically located in individual areas, lumps of intensively condensed chromatin were often visualized in their nuclei.

Focal destruction of the cells of the surface layer of the cornea and focal layering in such areas of fine-grained acidophilic masses of rice were noted in places. (Fig. 2, Fig. 3).

In most areas of the corneal stroma, the connective tissue plates were arranged in an orderly manner, and their collagen fibers were preserved. In some areas of the corneal stroma, connective tissue plates were swollen, their main substance was slightly swollen.

The loose arrangement of connective tissue plates, their delamination, and the accumulation of optically light masses in such areas were also noted (Fig. 1). The endothelium of the anterior chamber of the eye was preserved in most areas of the cornea.

However, in some areas of the cornea, swelling of the cytoplasm of cells of the posterior corneal epithelium was noted.

DISCUSSIONS

The most frequently diagnosed eye diseases is a group of corneal lesions of various origins, which have different etiology, pathogenesis, and clinical manifestations [3-8]. Among the main causes of their occurrence, various authors name injuries, bacterial lesions, as well as general metabolic changes in the body, which develop under the influence of both exo- and endogenous factors, as well as background of diseases that lead to the development of polyneuro- and polyangiopathies, in particular - the background of diabetes.

Chang Y.S. et al. (2020) and Badawi A.E. et al. (2017) in their works emphasize the significantly more frequent damage to the cornea by pathological processes in patients with diabetes than in the general population [6, 8].

The same opinion is held by Vieira-Potter V.J. et al. (2016), Wang B et al. (2018) [12, 13]. In order to understand the causes of diabetic keratopathies and to develop effective methods of their prevention and treatment, it is necessary to study in-depth the changes that occur in the structures of the cornea at the background of diabetes, starting from the early stages of its development. Understanding the mechanisms of their development will also allow to optimize and accelerate the processes of corneal regeneration after trauma or surgery in patients with diabetes.

The results of our study indicate that condition of the rat cornea influenced by experimental diabetes at the end of the second week of experiment showed the development of focal destruction of the corneal surface layer cells, which can be interpreted as the initial phenomena of diabetic keratitis. The detected changes explain why even at the initial stages of the development of this pathology, the examined tissues become more vulnerable to external stimuli, and in the absence of treatment (both general and local), these changes can progress and, as a result, cause the development of acute or chronic keratitis and significant decrease in visual acuity.

In order to preserve patients' vision and prevent the development of more serious complications, in our opinion, it is advisable to develop measures for early correction of detected changes already at the initial stages of the of diabetes development.

CONCLUSIONS

1. The result of our study of the cornea, at the end of the second week of experimental streptozotocin-induced diabetes found that in some areas of the cornea, the basal cells were located loosely, lost their columnar shape, and the cells of the basal layer with lightened vacuolated cytoplasm were visualized.
2. There were local zones where focal destruction of the cells of the surface layer of the cornea and focal layering of fine-grained acidophilic masses were noted. In some areas of the corneal stroma, connective tissue plates were swollen, their main substance was slightly swollen.

3. There were foci of swelling of the cytoplasm of cells of the posterior corneal epithelium. These pathomorphological manifestations at the end of the second week of experimental streptozotocin-induced diabetes indicate the initial phenomena of diabetic keratitis.

REFERENCES

1. Fong DS, Aiello L, Gardner T et al. Retinopathy in diabetes. Diabetes Care. 2004;27(1):S84-7. doi: 10.2337/diacare.27.2007.s84.
2. Cheung N, Wong T. Diabetic retinopathy and systemic vascular complications. Prog Retin Eye Res. 2008;27(2):161-76. doi: 10.1016/j.preteyeres.2007.12.001.
3. Liew G, Wong T, Mitchell P et al. Retinopathy predicts coronary heart disease mortality. Heart. 2009;95(5):391-4. doi: 10.1136/hrt.2008.146670.
4. Vijan S, Hofer T, Hayward R. Cost-utilityanalysis of screening intervals for diabetic retinopathy in patients with type 2 diabetes mellitus. JAMA. 2000;283(7):889-96. doi: 10.1001/jama.283.7.889.
5. Kempen J, O'Colmain B, Leske M et al. Eye Diseases Prevalence Research Group. The prevalence of diabetic retinopathy among adults in the United States. Arch. Ophthalmol. 2004;122(4):552-563. doi: 10.1001/archopht.122.4.552.
6. Chang Y, Tai M, Ho C et al. Risk of Corneal Ulcer in Patients with Diabetes Mellitus: A Retrospective Large-Scale Cohort Study. Sci. Rep. 2020;10(1):7388. doi: 10.1038/s41598-020-64489-0.
7. Wang B, Yang S, Zhai HL et al. A comparative study of risk factors for cornea linfection in diabetic and non-diabetic patients. Int. J. Ophthalmol. 2018;11(1):43-47. doi: 10.18240/ijo.2018.01.08.
8. Badawi AE, Moemen D, El-Tantawy NL. Epidemiological, clinical and laboratory findings of infectio us keratitisat Mansoura Ophthalmic Center, Egypt. Int. J. Ophthalmol. 2017;10(1):61-67. doi: 10.18240/ijo.2017.01.10.
9. Hovind P, Tarnow L, Rossing K. Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. Diabetes Care. 2003;26(4):1258-1264. doi: 10.2337/diacare.26.4.1258.
10. Nordwall M, Bojestig M, Arnqvist HJ. Diabetes Complications Study. Declining incidence of severe retinopathy and persisting decrease of nephropathy in anunse lected population of type 1 diabetes. Diabetologia. 2004;47(7):1266- 1272. doi: 10.1007/s00125-004-1431-6.
11. Bloomgarden Z.T, Drexler A. Fibrateuse in diabetes. Newconcepts. J. Diabetes. 2011;3(1):1-2. doi:10.1111/j.1753-0407.2010.00108.x.
12. Vieira-Potter VJ, Karamichos D, Lee DJ. Ocular complications of diabetes and therapeutica pproaches. Biomed Res Int. 2016:2016:3801570. doi: 10.1155/2016/3801570.
13. Markoulli M, Flanagan J, Tummanapalli SS et al. The impact of diabetes on corneal nerve morphology and ocular surface integrity. Ocul Surf. 2018;16(1):45-57. doi: 10.1016/j.jtos.2017.10.006.
14. Grzybowski A, Kanclerz P, Huerva V et al. Diabetes and Phacoemulsification Cataract Surgery: Difficulties, Risksand Potential Complications. J Clin Med. 2019;8(5):716. doi: 10.3390/jcm8050716.
15. Kaji Y. Prevention of diabetic keratopathy. Br J Ophthalmol. 2005;89(3):254–255. doi: 10.1136/bjo.2004.055541.
16. Chang YS, Tai MC, Ho CH et al. Risk of Corneal Ulcer in Patients with Diabetes Mellitus: A Retrospective Large-Scale Cohort Study. Sci Rep. 2020;10(1):7388. doi: 10.1038/s41598-020-64489-0.
17. Barsegian A, Lee J, Salifu MO, McFarlane SI. Corneal Neuropathy: An Underrated Manifestation of Diabetes Mellitus. J Clin Endocrinol Diabetes. 2018. doi: 10.29011/ JCED-111/100011.
18. Badawi AE, Moemen D, El-Tantawy NL. Epidemiological, clinical and laboratory findings of infectio us keratitisat Mansoura Ophthalmic Center, Egypt. Int. J. Ophthalmol. 2017;10(1):61-67. doi: 10.18240/ijo.2017.01.10.
19. Rabinovitch A, Suarez-Pinzon WL. Role of cytokines in the pathogenesis of autoimmune diabetes mellitus. Rev. Endocr. Metab. Disord. 2003;4(3):2919. doi: 10.1023/a:1025160614313.
20. Yamaguchi T, Calvacanti BM, Cruzat A et al. Correlation between human tear cytokine levels and cellular corneal changes in patients with bacterial keratitis by in vivo confocal microscopy. Invest. Ophthalmol. 2014;55(11):7457-66. doi: 10.1167/iovs.14-15411.
21. Konstantopoulos A, Cendra MM, Tsatsos M et al. Morphological and cytokine profiles as key parameters to distinguish between Gram-negative and Gram-positive bacterial keratitis. Sci Rep. 2020;10(1):20092. doi: 10.1038/s41598-020-77088-w.
22. Yao Y, Li R, Du J et al. Interleukin-6 and Diabetic Retinopathy: A Systematic Reviewand Meta-Analysis. Curr. Eye Res. 2019;44(5):564-74. doi: 10.1080/02713683.2019.1570274.
23. Bonyek-Silva I, Nunes S, Santos RL et al. Unbalancedproduction of LTB4/ PGE2 driven by diabetes increases susceptibility to cutaneous leishmaniasis. Emerg. Microbes Infect. 2020;9(1):1275-86. doi: 10.1080/22221751.2020.1773744.
24. Mehta P, Mc Auley DF, Brown M et al. HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-34. doi: 10.1016/ S0140-6736(20)30628-0.
25. Geerlings SE, Brouwer EC, VanKessel KC et al. Cytokinese cretionisimpaired in women with diabetes mellitus. Adv Exp Med Biol. 2000:485:255-62. doi: 10.1007/0-306-46840-9_34.

26. Yende S, Vander Poll T, Lee M. et al. The influence of pre-existing diabetes mellitus on the host immune response and outcome of pneumonia: analysis of two multicentre cohortstudies. Thorax. 2010;65(10):870-7. doi: 10.1136/thx.2010.136317. DOI

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Oleksandr M. Slobodian
Bukovinian State Medical University
2 Theatralna st, 58002 Chernivtsi, Ukraine
e-mail: slobodjanaleksandr@ukr.net

ORCID AND CONTRIBUTIONSHIP
Oleksandr M. Slobodian: 0000-0002-0444-8457 A B
Zoriana Z. Masna: 0000-0003-2057-7061 C F
Yaroslav I. Penishkevych: 0000-0003-2690-1352 F
Khrystyna I. Rudnytska: 0000-0001-7517-1515 D
Ilona V. Chelpanova: 0000-0001-5215-814X E
Olena V. Smolkova: 0000-0001-9474-7831 D
Kostyantyn I. Voytsenko: 0009-0003-5104-9509 B

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 20.06.2024
ACCEPTED: 29.09.2024