THE CORNEAL CHANGES IN DIABETIC PATIENTS

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PROMENE NA ROŽNJAJČI KOD PACIJENATA SA DIJABETESOM

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ABSTRACT

Diabetes mellitus (DM) represents a systemic disorder which affects different organs. Ocular complications of the DM are the worldwide leading cause of blindness. The most common complications are diabetic retinopathy, diabetic cataract, neovascular glaucoma. Recently many investigations point out that DM can cause complications at ocular surface as well. Condition such as decreased corneal sensitivity, dry eye or neurotrophic corneal ulceration are the main clinical manifestations of the diabetic keratopathy (DK). Untreated, these conditions can lead to serious visual acuity decrease. Pathological processes, based on chronic inflammation, due to chronic hyperglycemia, are the main step in the process of DK development. Adequate treatment of the main disease - DM is an imperative in maintaining the healthy cornea without subjective sensations of diabetic patients.

Keywords: diabetes mellitus, cornea, ocular surface, dry eye, corneal ulceration.

SAŽETAK

Dijabetes mellitus (DM) predstavlja sistemski poremećaj koji pogađa različite organe. Očne komplikacije DM-a vodeći su uzrok slepila u svetu. Najčešće komplikacije su dijabetesna retinopatija, dijabetesna katarakta, neovaskularni glaukom. U poslednje vreme mnoga istraživanja ukazuju se da DM može izazvati i komplikacije na površini oka. Stanje poput smanjene osetljivosti rožnjače, suvog oka ili neurotrofične ulceracije rožnjače glavne su kliničke manifestacije dijabetesne keratopatije (DK). Ako se ne leče, ova stanja mogu dovesti do ozbiljnog umanjenja vidne oštine. Patološki procesi, zasnovani na hroničnoj inflamaciji, usled hronične hiperglikemije, glavni su korak u procesu razvoja DK. Adekvatno lečenje osnovne bolesti - DM imperativ u održavanju zdrave rožnjače bez subjektivnih senzacija pacijenata sa dijabetesom.

Ključne reči: dijabetes melitus, površina oka, suvo oko, ulceracija rožnjač.

ABBREVIATIONS

AGE – glycation end-product
BM – basement membrane
CNTF – ciliary neurotrophic factor
CEBC – corneal epithelium basal cells
CCT – central corneal thickness
DK – diabetic keratopathy
DR – diabetic retinopathy
ECD – endothelial cell density

IL – interleukin
IVCM – in vivo confocal microscopy
LESC – limbal epithelial stem cell
LC – Langerhans cells
MMP – matrix metalloproteinase
ROS – reactive oxygen species
TIMP – tissue inhibitors of metalloproteinases

INTRODUCTION

Diabetic ocular complications are in correlation with increased prevalence of the diabetes mellitus in global population (1). Diabetic ocular complications - diabetic keratopathy (DK), diabetic cataract, dry eye and diabetic retinopathy (DR) can produce severe vision acuity deterioration and in final stadium irreversible blindness. Diabetic retinopathies as well as diabetic cataract are very investigated and lots facts are wellknown. Diabetic keratopathy is now very interesting for the future examination. Decreased or lost corneal sensitivity, dry eye or neurotrophic corneal ulceration are the main clinical manifestations of the DK.

NERVS CHANGES IN DIABETIC CORNEA

Chronic hyperglycemia induces damages of the trigeminal nerve, which causes decreased or lost corneal sensitivity. This ophthalmological condition is characterized with irritative symptoms (blepharospasm, epiphora, photophobia), and pain. These symptoms are the results of damage to the small A δ and C nerve fibers of the cornea (2). Decreased or loss corneal sensitivity trigger corneal epithelial breakdown with prolonged healing of the defects, and corneal ulceration, melting and perforation can be the final complications.

Diabetic patients have decreased length and density of the nerve fiber bundles with pronounced thickening in corneal sub-basal nerve plexus, which were documented by *in vivo* confocal microscopy (IVCM) (3) (4) (5). These findings are in correlation with the development of the DR. Patients with proliferative DR have statistically significant decreased length and density of the corneal nerves with greater thickness than patients without proliferative DR (6). The prediction of somatic neuropathy is in correlation with the tortuosity of the corneal sub-basal nerve (7). The condition of the stromal nerves is very hard to determine, due to poor instruments which can be used for that examination. Only immunofluorescence staining can record stromal nerve fiber loops. Patel and McGhee found that mean stromal nerve thickness and tortuosity are greater in patients with proliferative DR in comparison with patients with background form of the DR (8). Basement membrane becomes thicker and with increased resistance, due to hyperglycemia for entering of the stromal nerves in epithelium, with nerve loops forming (9).

Hyperglycemia induces increased production of glycation end-products (AGEs), reactive metabolites, which induce formation of retinal diabetic neuropathy (10) (11). Liberated AGEs with their receptors start inflammation process by releasing proinflammatory cytokines (12). Reactive oxygen species (ROS), as the result of the chronic hyperglycemia, induce the mitochondrial damages (13). These mitochondrial damages provoke demyelination and conduction dysfunction of nerve fibers (14). As the inflammation was accused as the main cause of the DK, some studied reported the increased proportion of dendritic and Langerhans cells (LCs), with positive correlation of LCs density (increased number) and severity of neuropathy (15).

Corneal nerve plexus has the main role in maintain the corneal homeostasis, so it provokes normal wound healing process by releasing neuropeptides, growth factors, and cytokines. In DK, levels of neuropeptides and ciliary neurotrophic factor (CNTF) are decreased with decreased number of dendritic cells (16) (17).

EPITHELIAL CHANGES IN DK

Corneal epithelium basal cells (CEBC), with role to protect inner parts of the cornea and the eye, consist of cell layers and the basement membrane. Diabetic patients have vulnerable corneal epithelium. Superficial punctate keratitis and corneal erosions are very common in those patients, and can be long-term complications of DM. Corneal epithelial basal cells, originated from limbal stem cells, have the main role in producing process of the basement membrane. They are presented as intermittently dark and bright cluster polygonal cells, with high reflective cell border. In patients with DM, IVCM noticed abnormal hypersignals in junction between the epithelium and anterior stroma. These phenomena can be attributed to accumulation of AGEs (18). Recent data reported significant decreased CCT in rat models with diabetes, especially in those with severe diabetic neuropathy (19) (20). Authors noticed decreased number of ECD, with diversity in their size and morphology in correlation with nerve brunch and nerve fiber density.

Disturbed innervations can be the main reason for the CEBC alterations in diabetic patients. So DK is provoked by abnormal production of the neuropeptides' molecules nerve growth factors and CNTF, which maintain corneal epithelial homeostasis (21) and promote corneal wound healing (22) (23). Some experimental studies noticed that addition of some neuropeptides can promote accelerated wound healing (24) (25).

BASEMENT MEMBRANE'S CHANGES IN DK

Corneal changes of diabetic patients are in correlation with the severity of diabetic disease. Taylor and Kimsey reported the increased thickness of the corneal basement membrane, which they detected using the electron microscopy. Their results implicated that the corneal changes correlated with the severity of DM, and can be useful for the early detection of the DM (26). Changes in corneal BM can be explained by CEBC layer reduction with disturbed function of hemidesmosomes, decreased expression of some fibrous tissue components and abnormal basal membrane metabolism (27). MMP activity is also very interesting in diabetic cornea. For example, MMP-10/stromelysin-2 promotes proteolytic degradation of basement membrane components in DM, as well as the activation of MMP-9, which decreases the interaction of type IV collagen with other proteins in cell junction process (28) (29).

Abnormal production and accumulation of the AGE have, also, very important role in the DK pathogenesis. Elevated corneal autofluorescence was recorded in patients with DM,

especially in corneal epithelium and basement membrane junction, with apoptotic activity to epithelial cells (30) (31). These changes were in correlation with severity of DM (32).

STROMAL CHANGES IN DK

DM provokes the corneal stromal structural and functional modifications, with consequential visual acuity deterioration (33). Reachable data suggested that CCT is increased in diabetic patients in correlation with the intensity of the diabetic peripheral neuropathy, due to the stromal changes (34). Anterior corneal stromal organization was different in various parts of the cornea: central cornea showed normal structural organization with thicker basal epithelial lamina, but peripheral cornea had tile shaped collagen fibril in epithelial basal lamina (35). Some experimental studies implicated that decreased corneal transparency was due to abnormal collagen fibril bundles with diverse thickness and intercellular space, and increased AGE immune reactivity. Increased AGE immune reactivity provokes collagen crosslinking with stromal alterations (36). Accumulated ROS with increased levels of some growth factors activate proliferation and activation of keratocytes in posterior stroma in young diabetic patients (6) (37) (38).

Balance between the MMPs and tissue inhibitors of metalloproteinases (TIMPs) is disturbed in patients with DM. Upregulated activities of MMP-3 and MMP-10 and down-regulated activity of TIMPs-4 are also included in the process of stromal alterations (39).

Chronic hyperglycemia provokes the strengthening of adhesion of the posterior stroma and Descemet membrane (40). Some novel biomarkers were detected in corneal stroma (aminoadipic acid, pipecolic acid, and dihydroorotate) of diabetic patients. Those biomarkers may be the results of the chronic hyperglycemia (41).

ENDOTHELIAL CHANGES IN DK

Patients with DM had alterations in endothelial layer, such as: lower mean density of endothelial cell (ECD), down-regulated recovery process of endothelial cells with consequential lower percentage of hexagonal cells and with increased CCT (31) (42) (43). Functional abnormalities were due to impairment of corneal dehydration with consequential swelling and increased CCT. This condition is in relation with increased endothelial permeability and autofluorescence (44). Diabetic patients with higher Hemoglobin A1c level had decreased ECD (45) (46). Also, polymegethism and pleomorphism are very common in eyes of the patients with DM, and is in tight junction with the previous described changes in cornea-increased CCT, low ECD (47). Detailed immunological examination of the endothelial layer of cornea recorded the presence of some inflammatory factors - vascular endothelial growth factor, tumor necrosis factor- α , interleukin (IL), and MMP. Those factors may cause functional and morphological changes in corneal endothelia, and the endothelial recovery process gets slower and weaker (48).

Chronic hyperglycemia provokes non-enzymatic glycosylation of proteins with sorbitol accumulation. Accumulated AGEs promote decreased ECD with altered metabolism (49). Metabolic endothelial changes include mitochondrial dysfunction with accumulation of ROS, and mitochondrial injury (50).

OTHER CHANGES IN DK

Detailed examination of Descemet membrane indicated for the presence of long-spacing collagen fibril, due to the accumulation of AGEs which were detected on Descemet membrane in diabetic rat model (51) (52). In experimental condition long-spacing collagen fibril production can be blocked with the application of antidiabetic agents (53).

Corneal limbal epithelial stem cells (LESCs) are narrow band that encircles the cornea, with great proliferative potential, especially for the process of corneal wound healing. IVCN examination of these LESCs showed loss of the regulatory of limbal epithelium, with intraepithelial cysts and mosaic pattern of cells with great morphological diversity (54). Corneal damage may be warned using insulin-like growth factor-1, as some experts suggested (55).

The main role of the tear film is to keep morphological and functional integrity of the cornea. Lacrimal system functions if every its elements work correctly as entirety: lacrimal glands, lacrimal drainage system and innervations system. Dry eye syndrome is very common condition described in connection to diabetes mellitus (56) (57). Dry eye syndrome developed if some of the lacrimal system elements do not function well. Dry eye syndrome in diabetic patients can be represented as: superficial punctate keratopathy, secondary bacterial infection, and even perforation. The most common reason for dry eye syndrome in diabetic patients is dysfunction of the lacrimal glands (58).

Chronic hyperglycemia provokes the releasing of the pro-inflammatory cytokines (IL-1 α , IL-1 β , IL-6, and tumor necrosis factor- α) on the ocular surface (59) (60). Chronic inflammatory process is the main pathological process. As we mentioned above, MMP-9 as an important inflammatory mediator, is in tight conjunction with ocular surface inflammation in diabetic patients (61). Substance P, as an inflammatory marker, can be decreased in the tears of patients with diabetes mellitus (16). Experimentally studies detected disturbed oxidative status in lacrimal gland acinar cells (62).

One of the leading reasons for the dry eye syndrome is the hyperosmolarity of the tear. Tear film hyperosmolarity is very common described as the complication of the chronic hyperglycemia. Hyperosmolarity of the tears additionally provoke surface inflammation with increased evaporation of the water layer of the tear film. By this way vicious cycle can be made (63).

Disturbed corneal innervations, due to diabetic sensory nerve impairment, also provoke disturbed tear film secretion

(64). Hyper evaporation of the tear can be also in connection with the dysfunction of the meibomian gland. Hyperglycemia aggravates the function of the epithelial cells of the meibomian glands (65).

CONCLUSION

DM is very common described as the condition which may cause different ocular manifestations. Diabetic keratopathy is now described as one of them. Different pathological processes, based on chronic inflammation due to chronic hyperglycemia, are presumably the main step in the process of DK development. Adequate treatment of the main disease - DM is imperative if we want to have health cornea without subjective sensations of our diabetic patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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LITERATURE

- Threatt J, Williamson JF, Huynh K, Davis RM, Her-mayer K. Ocular disease, knowledge and technology applications in patients with diabetes. *Am J Med Sci*. 2013;345(4):266-270.
- Bikbova G, Oshitari T, Baba T, Bikbov M, Yamamoto S. Diabetic corneal neuropathy: clinical perspectives. *Clin Ophthalmol*. 2018;12:981-987.
- Rosenberg ME, Tervo TM, Immonen IJ, Müller LJ, Grön-hagen-Riska C, Vesaluoma MH. Corneal structure and sensitivity in type 1 diabetes mellitus. *Invest Oph-thalmol Vis Sci*. 2000;41(10):2915-2921.
- Mocan MC, Durukan I, Irkeç M, Orhan M. Morphologic alterations of both the stromal and subbasal nerves in the corneas of patients with diabetes. *Cornea*. 2006;25(7):769-773.
- Edwards K, Pritchard N, Vagenas D, Russell A, Malik RA, Efron N. Utility of corneal confocal microscopy for assessing mild diabetic neuropathy: baseline findings of the LANDMark study. *Clin Exp Optom*. 2012;95(3):348-354.
- Szalai E, Deák E, Módis L Jr, Németh G, Berta A, Nagy A, Felszeghy E, Káposzta R, Malik RA, Csutak A. Early corneal cellular and nerve fiber pathology in young pa-tients with type 1 diabetes mellitus identified using cor-neal confocal microscopy. *Invest Oph. Vis Sci*. 2016;57(3):853-858
- Kallinikos P, Berhanu M, O'Donnell C, Boulton AJ, Efron N, Malik RA. Corneal nerve tortuosity in diabetic patients with neuropathy. *Invest Ophthalmol Vis Sci*. 2004;45(2):418-422.
- Patel DV, McGhee CN. In vivo confocal microscopy of human corneal nerves in health, in ocular and systemic disease, and following corneal surgery: a review. *Br J Ophthalmol*. 2009;93(7):853-860.
- He JC, Bazan HE. Mapping the nerve architecture of di-abetic human corneas. *Ophthalmology*. 2012;119(5):956-964.
- Madonna R, Balistreri CR, Geng YJ, De Caterina R. Di-abetic microangiopathy: Pathogenetic insights and novel therapeutic approaches. *Vascul Pharmacol*. 2017;90:1-7.
- Bikbova G, Oshitari T, Baba T, Yamamoto S. Mecha-nisms of neuronal cell death in AGE-exposed retinas-re-search and literature review. *Curr Diabetes Rev*. 2017;13(3):280-288.
- Kim J, Kim CS, Sohn E, Jeong IH, Kim H, Kim JS. In-volvement of advanced glycation end products, oxida-tive stress and nuclear factor-kappaB in the development of diabetic keratopathy. *Graefes Arch Clin Exp Ophthal-mol*. 2011;249(4):529-536.
- Babizhayev MA, Stokov IA, Nosikov VV, Savel'yeva EL, Sitnikov VF, Yegorov YE, Lankin VZ. The role of oxidative stress in diabetic neuropathy: generation of free radical species in the glycation reaction and gene polymorphisms encoding antioxidant enzymes to ge-netic susceptibility to diabetic neuropathy in population of type I diabetic patients. *Cell Biochem Biophys*. 2015;71(3):1425-1443.
- Yagihashi S, Mizukami H, Sugimoto K. Mechanism of diabetic neuropathy: Where are we now and where to go? *J Diabetes Investig*. 2011;2(1):18-32.
- Leppin K, Behrendt AK, Reichard M, Stachs O, Guthoff RF, Baltrusch S, Eule JC, Vollmar B. Diabetes mellitus leads to accumulation of dendritic cells and nerve fiber damage of the subbasal nerve plexus in the Cornea. *Invest Ophthalmol. Vis Sci* 2014;55(6): 3603-3615.
- Markoulli M, You JJ, Kim J, Duong CL, Tolentino JB, Karras J, Lum E. Corneal nerve morphology and tear film substance P in diabetes. *Optom Vis Sci*. 2017;94(7):726-731.
- Dana MR. Corneal antigen-presenting cells: diversity, plasticity, and disguise: the Cogan lecture. *Invest Oph-thalmol Vis Sci*. 2004;45(3):722-727; 721.
- Qu JH, Li L, Tian L, Zhang XY, Thomas R, Sun XG. Epithelial changes with corneal punctate epitheliopathy in type 2 diabetes mellitus and their correlation with time to healing. *BMC Ophthalmol*. 2018;18(1):1.
- Cai D, Zhu MF, Petroll WM, Koppaka V, Robertson DM. The impact of type 1 diabetes mellitus on corneal epithelial nerve morphology and the corneal epithelium. *Am J Pathol*. 2014;184(10):2662-2670.
- Chang PY, Carrel H, Huang JS, Wang IJ, Hou YC, Chen WL, Wang JY, Hu FR. Decreased density of corneal ba-sal epithelium and subbasal corneal nerve bundle changes in patients with diabetic retinopathy. *Am J Oph-thalmol*. 2006;142(3):488-490.
- Di GH, Qi X, Zhao XW, Zhang SM, Danielson P, Zhou QJ. Corneal epithelium-derived neurotrophic factors promote nerve regeneration. *Invest Ophthalmol Vis Sci*. 2017;58(11):4695-4702.

22. Zhou QJ, Chen P, Di GH, Zhang YY, Wang Y, Qi X, Duan HY, Xie LX. Ciliary neurotrophic factor promotes the activation of corneal epithelial stem/progenitor cells and accelerates corneal epithelial wound healing. *Stem Cells*. 2015;33(5):1566-1576.
23. Park JH, Kang SS, Kim JY, Tchah H. Nerve growth factor attenuates apoptosis and inflammation in the diabetic Cornea. *Invest Ophthalmol Vis Sci*. 2016;57(15):6767-6775.
24. Morishige N, Uemura A, Morita Y, Nishida T. Promotion of corneal epithelial wound healing in diabetic rats by the fibronectin-derived peptide PHSRN. *Cornea*. 2017;36(12):1544-1548.
25. Hu JZ, Hu XY, Kan T. MiR-34c participates in diabetic corneal neuropathy via regulation of autophagy. *Invest Ophthalmol Vis Sci*. 2019;60(1):16-25.
26. Taylor HR, Kimsey RA. Corneal epithelial basement membrane changes in diabetes. *Invest Ophthalmol Vis Sci*. 1981;20(4):548-553.
27. Ljubimov AV, Huang ZS, Huang GH, Burgeson RE, Gullberg D, Miner JH, Ninomiya Y, Sado Y, Kenney MC. Human corneal epithelial basement membrane and integrin alterations in diabetes and diabetic retinopathy. *J Histochem Cytochem*. 1998;46(9):1033-1041.
28. Tomomatsu T, Takamura Y, Kubo, Akagi Y. Aldose reductase inhibitor counteracts the attenuated adhesion of human corneal epithelial cells induced by high glucose through modulation of MMP-10 expression. *Diabetes Res Clin Pract*. 2009;86(1):16-23.
29. Takahashi H, Akiba K, Noguchi T, Ohmura T, Takahashi R, Ezure Y, Ohara K, Zieske JD. Matrix metalloproteinase activity is enhanced during corneal wound repair in high glucose condition. *Curr Eye Res*. 2000;21(2):608-615.
30. Ishida M, Yokoi N, Okuzawa J, Maeda K, Kinoshita S. Corneal autofluorescence in patients with diabetic retinopathy. *Nippon Ganka Gakkai Zasshi*. 1995;99(3):308-311.
31. Mishima S. Clinical investigations on the corneal endothelium. *Ophthalmology*. 1982;89(6):525-530.
32. Sato E, Mori F, Igarashi S, Abiko T, Takeda M, Ishiko S, Yoshida A. Corneal advanced glycation end products increase in patients with proliferative diabetic retinopathy. *Diabetes Care*. 2001;24(3):479-482.
33. Torricelli AA, Wilson SE. Cellular and extracellular matrix modulation of corneal stromal opacity. *Exp Eye Res*. 2014;129:151-160.
34. Kumar N, Pop-Busui R, Musch DC, Reed DM, Momont AC, Hussain M, Raval N, Moroi SE, Shtein R. Central corneal thickness increase due to stromal thickening with diabetic peripheral neuropathy severity. *Cornea*. 2018;37(9):1138-1142.
35. Kowalczyk L, Latour G, Bourges JL, Savoldelli M, Jeanny JC, Plamann K, Schanne-Klein MC, Behar-Cohen F. Multimodal highlighting of structural abnormalities in diabetic rat and human corneas. *Transl Vis Sci Technol*. 2013;2(2):3.
36. Zou CL, Wang SY, Huang F, Zhang YA. Advanced glycation end products and ultrastructural changes in corneas of long-term streptozotocin-induced diabetic monkeys. *Cornea*. 2012;31(12):1455-1459.
37. Priyadarsini S, Rowsey TG, Ma JX, Karamichos D. Unravelling the stromal-nerve interactions in the human diabetic Cornea. *Exp Eye Res*. 2017;164:22-30.
38. Todorovic D, Sarenac Vulovic T, Sreckovic S, Jovanovic S, Janicijevic K, Todorovic Z. Updates on the treatment of pterygium. *Ser J Exp Clin Res*. 2016; 17 (3): 257-261 doi: 10.1515/SJECR-2016-0012.
39. Saghizadeh M, Kramerov AA, Tajbakhsh J, Aoki AM, Wang C, Chai NN, Ljubimova JY, Sasaki T, Sosne G, Carlson MR, Nelson SF, Ljubimov AV. Proteinase and growth factor alterations revealed by gene microarray analysis of human diabetic corneas. *Invest Ophthalmol*. 2005;46(10):3604-3615.
40. Schwarz C, Aldrich BT, Burckart KA, Schmidt GA, Zimmerman MB, Reed CR, Greiner MA, Sander EA. Descemet membrane adhesion strength is greater in diabetes with advanced disease compared to healthy donor corneas. *Exp Eye Res*. 2016;153:152-158.
41. Priyadarsini S, McKay TB, Sarker-Nag A, Allegood J, Chalfant C, Ma JX, Karamichos D. Complete metabolome and lipidome analysis reveals novel biomarkers in the human diabetic corneal stroma. *Exp Eye Res*. 2016;153:90-100.
42. Liaboe CA, Aldrich BT, Carter PC, Skeie JM, Burckart KA, Schmidt of diabetes mellitus on donor corneal endothelial cell density. *Cornea*. 2017;36(5):561-566.
43. El-Agamy A, Alsubaie S. Corneal endothelium and central corneal thickness changes in type 2 diabetes mellitus. *Clin Ophthalmol*. 2017;11: 481-486.
44. Sahu PK, Das GK, Agrawal S, Kumar S. Comparative evaluation of corneal endothelium in patients with diabetes undergoing phacoemulsification. *Middle East Afr J Ophthalmol*. 2017;24(2):74-80.
45. Storr-Paulsen A, Singh A, Jeppesen H, Norregaard JC, Thulesen J. Corneal endothelial morphology and central thickness in patients with type II diabetes mellitus. *Acta Ophthalmol* 2014;92(2):158-160.
46. Shenoy R, Khandekar R, Bialasiewicz A, Al Muniri A. Corneal endothelium in patients with diabetes mellitus: a historical cohort study. *Eur J Ophthalmol*. 2009;19(3):369-375.
47. González-Méijome JM, Jorge J, Queirós A, Peixoto-de-Matos SC, Parafita MA. Two single descriptors of endothelial polymegethism and pleomorphism. *Graefes Arch Clin Exp Ophthalmol*. 2010;248(8):1159-1166.
48. Mathew PT, David S, Thomas N. Endothelial cell loss and central corneal thickness in patients with and without diabetes after manual small incision cataract surgery. *Cornea*. 2011;30(4):424-428.
49. Kaji Y, Amano S, Usui T, Suzuki K, Tanaka S, Oshika T, Nagai R, Horiuchi S. Advanced glycation end products in Descemet's membrane and their effect on corneal endothelial cell. *Curr Eye Res*. 2001;23(6):469-477.
50. Aldrich BT, Schlötzer-Schrehardt U, Skeie JM, Burckart KA, Schmidt GA, Reed CR, Zimmerman MB, Kruse FE, Greiner MA. Mitochondrial and morphologic alterations in native human corneal endothelial cells associated with

- diabetes mellitus. *Invest Ophthalmol Vis Sci*. 2017;58(4):2130-2138
51. Eghrari AO, Riazuddin SA, Gottsch JD. Overview of the cornea: structure, function, and development. *Prog Mol Biol Transl Sci*. 2015;134: 7-23.
 52. Latour G, Kowalczyk L, Savoldelli M, Bourges JL, Plamann K, Behar-Cohen F, Schanne-Klein MC. Hyperglycemia-induced abnormalities in rat and human corneas: the potential of second harmonic generation microscopy. *PLoS One*. 2012;7(11):e48388.
 53. Akimoto Y, Sawada H, Ohara-Imaizumi M, Nagamatsu S, Kawakami H. Change in long-spacing collagen in Descemet's membrane of diabetic Goto-Kakizaki rats and its suppression by antidiabetic agents. *Exp Diabetes Res*. 2008;2008:818341.
 54. Nubile M, Lanzini M, Miri A, Pocobelli A, Calienno R, Curcio C, Mastropasqua R, Dua HS, Mastropasqua L. In vivo confocal microscopy in diagnosis of limbal stem cell deficiency. *Am J Ophthalmol*. 2013;155(2):220-232.
 55. Ueno H, Hattori T, Kumagai Y, Suzuki N, Ueno S, Takagi H. Alterations in the corneal nerve and stem/progenitor cells in diabetes: preventive effects of insulin-like growth factor-1 treatment. *Int J Endocrinol*. 2014;2014:312401.
 56. Stuard WL, Titone R, Robertson DM. Tear levels of insulin-like growth factor binding protein 3 correlate with subbasal nerve plexus changes in patients with type 2 diabetes mellitus. *Invest Ophthalmol Vis Sci*. 2017;58(14):6105-6112.
 57. Sarenac Vulovic T, Pavlovic S, Janicijevic K, Todosrovic D, Lutovac M, Paunovic S, et al. Tear film stability in patients with pseudexfoliation. *Ser J Exp Clin Res*. 2018; 19 (3): 243-246. doi: 10.1515/SJECR-2017-0002.
 58. Rocha EM, Alves M, Rios JD, Dartt DA. The aging lacrimal gland: changes in structure and function. *Ocul Surf*. 2008;6(4):162-174.
 59. Joussen AM, Poulaki V, Le ML, Koizumi K, Esser C, Janicki H, Schraermeyer U, Kociok N, Fauser S, Kirchhof B, Kern TS, Adamis AP. A central role for inflammation in the pathogenesis of diabetic retinopathy. *FASEB J*. 2004;18(12):1450-1452.
 60. Massingale ML, Li XH, Vallabhajosyula M, Chen DM, Wei Y, Asbell PA. Analysis of inflammatory cytokines in the tears of dry eye patients. *Cornea*. 2009;28(9):1023-1027.
 61. Kaufman HE. The practical detection of MMP-9 diagnoses ocular surface disease and may help prevent its complications. *Cornea*. 2013; 32(2):211-216.
 62. Liu H, Sheng M, Liu Y, Wang P, Chen Y, Chen L, Wang W, Li B. Expression of SIRT1 and oxidative stress in diabetic dry eye. *Int J Clin Exp Pathol*. 2015;8(6):7644-7653.
 63. Eissa IM, Khalil NM, El-Gendy HA. A controlled study on the correlation between tear film volume and tear film stability in diabetic patients. *J Ophthalmol*. 2016;2016:5465272.
 64. Cousen P, Cackett P, Bennett H, Swa K, Dhillon B. Tear production and corneal sensitivity in diabetes. *J Diabetes Complicat*. 2007;21(6):371-373.
 65. Ding J, Liu Y, Sullivan DA. Effects of insulin and high glucose on human meibomian gland epithelial cells. *Invest Ophthalmol Vis Sci*. 2015;56(13):7814-7820.
 66. Sayin N, Kara N, Pekel G. Ocular complications of diabetes mellitus. *World J Diabetes*. 2015; 6(1): 92-108.