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The effect of vitamin D and melatonin on the ocular tissues in streptozotocin – Induced diabetes model in rats

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Abstract. – OBJECTIVE: The aim of the study was to investigate the effectiveness of vitamin D and melatonin on the ocular tissues in streptozotocin (STZ)-induced diabetes.

MATERIALS AND METHODS: In this study, a total of 45 male Wistar rats were randomly divided into five groups as follows: 1) non-diabetic rats (control group); 2) untreated STZ-induced diabetic rats; 3) STZ-induced diabetic rats treated with vitamin D; 4) STZ-induced diabetic rats treated with melatonin; 5) STZ-induced diabetic rats treated with the combination of vitamin D and melatonin. After six weeks of treatment, all rats were sacrificed for post-mortem analyses. Retinal and corneal samples were obtained and analyzed for glial fibrillary acidic protein (GFAP) expression. Retinal and corneal thicknesses in addition to morphological changes were assessed via hematoxylin and eosin staining.

RESULTS: Untreated diabetic rats revealed retinal disorganization, atrophy, increased vascularization along with more GFAP staining. However, all the treated groups exhibited more regular retinal layers and minimal GFAP staining. Additionally, treatment groups showed more uniform corneal layers with minimal GFAP staining.

CONCLUSIONS: Melatonin and vitamin D could be used as an additional complementary treatment in diabetes. Developing treatment protocols involving supplementation, as well as informing patients about the potential benefits of vitamin D and melatonin could be impactful in the treatment process of diabetes.

Key Words:
Retinal inflammation, Corneal inflammation, Vitamin D, Melatonin.

Introduction

The pathogenesis of diabetic injury is believed to involve oxidative stress and hyperglycemia but most of its aspects are still under investigation[1]. Hyperglycemia-induced oxidative stress is one of the most common pathological mechanisms in diabetes mellitus, and it results in various complications, including diabetic keratopathy and retinopathy[1]. Also, the increased inflammatory process is another important factor responsible for diabetic damage[2]. In uncontrolled diabetes, oxidative stress leads to free radicals to accumulate, and the retina is highly susceptible to oxidative damage caused by these free radicals[2]. Oxidative stress also causes an increase in vascular endothelial growth factor (VEGF) levels in the diabetic retina[3]. Melatonin, a strong antioxidant and anti-inflammatory agent, is mainly synthesized and released from photoreceptors in the retina[4]. As well as being an effective antioxidant, it is also considered to be a direct free radical scavenger and stimulates several antioxidative enzymes[5]. There are some studies[2,6] affirming that melatonin reduces retinal VEGF levels. An alternative treatment method is vitamin D, which plays an important role in normal insulin release and maintenance of glucose tolerance. There is ample evidence suggesting that vitamin D has a role in insulin secretion, which includes the presence of the 1,25(OH)2D3 receptor in β cells and vitamin D-dependent calcium-binding proteins in pancreatic tissue[7,8]. Furthermore, vitamin D can decrease nitric oxide production[9], inhibit VEGF[10], downregulate the IGF-I pathway[11], and upregulate the cellular transporters of sulfate[12]. All these molecules have been implicated in the pathogenesis of DR. Similarly, calcium homeostasis and calcium-induced signal pathways have an important role in the development of retinal hypoxia[13].

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein present in numerous cell types including muller cells and supports the mechanical strength of astrocytes[14]. Increased

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GFAP production is a key indicator of glial reactivity[15]. Likewise, it is a biomarker for the process of immune inflammation[16]. The oxidative stress that occurs in DM causes dysfunction and structural abnormalities in Müller cells and upregulation of GFAP[17]. In addition to the central nervous system (brain, spinal cord, retina), GFAP has been observed in corneal stromal cells. However, there is limited information in the literature regarding corneal GFAP. In few studies[18,19], an increase in the corneal GFAP has been noted yet the mechanism is unclear.

So, in this study, we aim to evaluate the effectiveness of vitamin D and melatonin treatment, separately and in combination, on retinal inflammation and structural changes in diabetic rats.

Materials and Methods

In this study, a total of 45 male Wistar rats (10 weeks old) weighing between 200 and 250 grams were divided into 5 groups of 9 rats each. The animals were housed in well-ventilated 750 cm^2 polypropylene cages, each containing a maximum of 3 rats, in a room at 20-25°C with 60% humidity and 12 h light/dark cycle, with standard rat pellets and ad libitum access to water. They were treated in accordance with the Association for Research in Vision and Ophthalmology's Statement for the Use of Animals in Ophthalmic and Vision Research. The study protocol was approved by the Institutional Animal Care and Use Committee. Streptozotocin (STZ), an antibiotic produced by Streptomyces achromogenes, was used to induce experimental diabetes.

The animals were randomly divided into 5 groups as follows:

1. Control group: non-diabetic rats (CG);
2. Untreated diabetic rats: STZ-induced diabetes, no treatment;
3. STZ-induced diabetic rats treated with vitamin D (STZ+D);
4. STZ-induced diabetic rats treated with melatonin (STZ+M);
5. STZ-induced diabetic rats treated with combination vitamin D and melatonin (STZ+D/M).

Diabetes was induced in groups 2-5 using a single dose of 60 mg/kg STZ dissolved in 0.01 M citrate buffer pH 4.5 injected intraperitoneally. Rats in the control group received a single intraperitoneal injection of 0.9% NaCl.

At 72 hours after STZ injection, a blood sample was taken from the tail artery of each rat to diagnose diabetes. Rats with fasting blood glucose (FBG) levels of 250 mg/dl or higher were accepted as diabetic. For the next 6 weeks, the rats in groups 3 and 5 were treated with intraperitoneal vitamin D at a dose of 0.5 µg/kg/day and the rats in groups 4 and 5 were treated with intraperitoneal melatonin 10 mg/kg/day (Sigma-Aldrich, M5250, St. Louis, MO, USA) which was dissolved in 1% ethanol. Melatonin injections were performed daily in the evening, between 20:00 pm-22:00 pm. FBG in the diabetic rats was measured at 2-week intervals. At the end of 6 weeks, the rats were killed by cardiac puncture under ether anesthesia and transcardially perfused with heparinized saline followed by 10% formalin in phosphate buffer. Retinas and corneas were obtained from the rats by an ophthalmologist, subsequently stored at -70°C until analysis. For histopathological examinations, corneal and retinal specimens taken from the rats were fixed in 10% formalin solution and embedded in paraffin. Two slides of 4-µm thick corneal and retinal sections were prepared for analysis using the fully automated Leica ASP300S. For each tissue type, one slide was stained with hematoxylin and eosin and the other was stained with anti-GFAP antibody (Dako, clone 6F2, ready-to-use) using a Dako Autostainer 48 Link immunohistochemical staining machine. Retina and corneal thickness were measured (in µm) from the hematoxylin-stained retinal tissue using Olympus image analysis software (DP21). The proportion of GFAP-positive cells was calculated based on a count of 100 cells in the most intensely stained area and graded as 0 (no stained cells), 1 (1-5% stained cells), 2 (6-10% stained cells), or 3 (>11% stained cells).

Statistical Analysis

Data were reported as mean ± standard deviation. All data were analyzed using the nonparametric Kruskal-Wallis test, and subsequent individual comparisons were performed by Mann-Whitney U-test. The significance level was set at a *p*-value of < 0.05.

Results

Retinal GFAP and Histopathological Results

The retinal layers showed normal organization in the control group (Figure 1a). Retinal GFAP staining was significantly intensified in

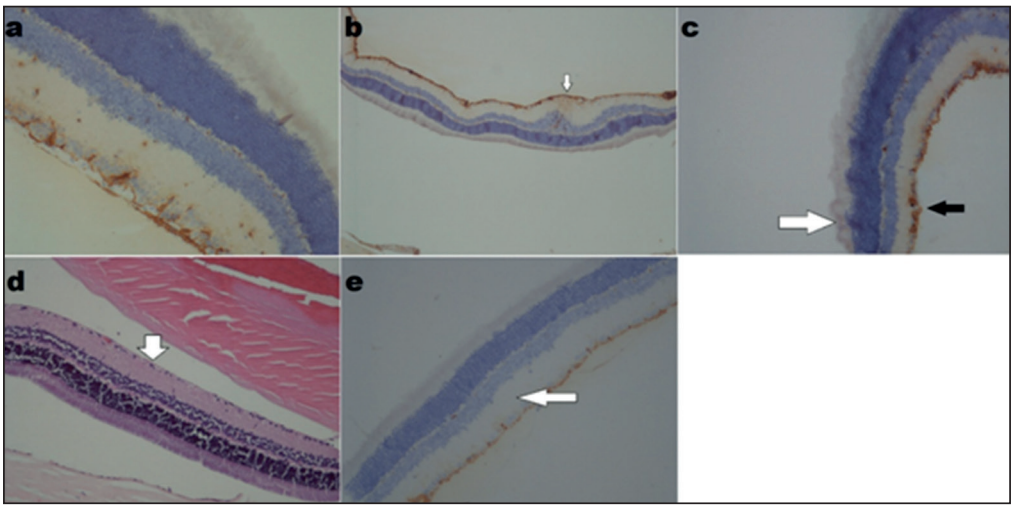

Figure 1. Example retinal sections from the study groups. (**a**) GFAP staining in retinal stroma and regular stratification in control group (x 100); (**b**) Retinal irregularity and GFAP positivity of gliosis area (arrow) in diabetic rats (X 100); (**c**) Retinal atrophy (white arrow) and 2 (+) GFAP staining (black arrow) in diabetic rats (x 100); (**d**) Regular retinal stratification in treated rats (arrow) (H&E x 100); (**e**) 1 (+) GF.

untreated diabetes (STZ) group compared to the control group. Correspondingly, in the untreated diabetes group, the retina appeared to be disorganized, with irregular and atrophied layers (Figure 1b, 1c).

In all treatment groups, the retinal layers appeared normal and there was minimal GFAP staining (Figure 1d, 1e). The STZ+D, STZ+M, and STZ+D/M groups had significantly less retinal GFAP staining compared to the untreated diabetic group. There was also less GFAP staining in the STZ+D group than the STZ+M group, and even less in the STZ+D/M group than the other two groups (Table I). However, retinal GFAP staining did not differ significantly between rats in the STZ+D, STZ+M, and STZ+D/M groups (Table I).

Corneal GFAP and Histopathological Results

Corneal samples taken from the control group and all treatment groups showed regular layers with minimal GFAP staining (Figure 2a, 2b, 2c, 2d). However, in the untreated diabetic group, corneal irregularity, vascularization, and more stromal GFAP staining were observed (Figure 2e). Untreated diabetic rats had the highest rate of corneal GFAP staining, while rats in the STZ+D/M group showed the least GFAP staining. Corneal GFAP staining was significantly lower in the STZ+D, STZ+M, and STZ+D/M groups compared to the untreated diabetic group (Table I).

Discussion

Despite the various medications and surgical methods, millions of people worldwide persistently develop sight-threatening DR. Therefore, receiving supportive treatment in the early stages, prior to the progression of the disease is crucial. On this subject, a great deal of work has been done by researchers. However, there is no prior information on the combined use of vitamin D and melatonin.

In a study conducted by Jiang et al[2], melatonin reduced the amount of VEGF, thus they stated that melatonin has a protective role for diabetic retinopathy. Baydas et al[20] reported that reactive gliosis was prevented by melatonin administration. Ozdemir et al[21] showed that melatonin diminished the number of molecules responsible for oxidative damage, such as nitrotyrosine and malondialdehyde, as well as the VEGF level which is responsible for vascular changes and microvasculopathy.

Similarly, we found that melatonin treatment either alone or in combination with vitamin D had a significant effect on retinal inflammation. Also, combination treatment had better results.

Vitamin D treatment has been shown to be effective in the treatment and prevention of diabetes mellitus in both human[22] and animal models[23]. Many studies[24-26] have reported an inverse relationship between vitamin D deficiency (VDD) and DR. Aksoy et al[27] reported an association between lower levels of active vitamin D (1,25-dihyroxyvitamin D3) and increased retinopathy. Sim-

Table I. Comparisons of retinal and corneal GFAP staining between groups.

Groups		n (%)	Retinal GFAP G.Median (Min. / Max.)	Corneal GFAP G.Median (Min. / Max.)
1 (control)		9 (20)	1.6 (1 / 2)	0.7 (0 / 2)
2 (STZ)		9 (20)	2.4 (2 / 3)	1.9 (1 / 3)
3 (STZ+D)		9 (20)	1.3 (1 / 2)	0.7 (0 / 2)
4 (STZ+M)		9 (20)	1.5 (0 / 2)	0.7 (0 / 2)
5 (STZ+D/M)		9 (20)	1.1 (0 / 2)	0.6 (0 / 2)
Total		**45 (100)**	**1.6 (0 / 3)**	**0.9 (0 / 3)**
***p*-value**			**<0.001**	**0.015**
Pairwise Comparisons	**1→2**		**0.013***	**0.010***
	1→3		0.444	1.000
	1→4		0.837	0.962
	1→5		0.176	0.744
	2→3		**0.001***	**0.010***
	2→4		**0.007***	**0.009***
	2→5		**<0.001***	**0.004***
	3→4		0.576	0.962
	3→5		0.556	0.744
	4→5		0.251	0.781

Kruskal-Wallis Test (Monte Carlo) - Post Hoc Test: Dunn's Test, G. Median: Grouped Median, Min.: Minimum, Max.: Maximum, * statistical significant ($p<0.05$).

ilarly, Ashinne et al[28] demonstrated that lower serum 25(OH)D was associated with increased severity of DR, and the presence of vitamin D deficiency was associated with a two-fold increased risk of proliferative DR. Furthermore, Albert et al[29] showed calcitriol (1α,25-dihydroxyvitamin D3) has an inhibitory effect on retinal neovascularization, which may also be beneficial in the treatment of ocular diseases with the neovascular component. In contrast, some studies[30,31] in the literature failed to detect an association between vitamin D and DR. Patrick et al[32] found a correlation between the severity of diabetic retinopathy and prevalence of VDD, but they declared that findings were inconclusive about the existence of a relationship between retinopathy severity and serum 25-hydroxyvitamin D concentration.

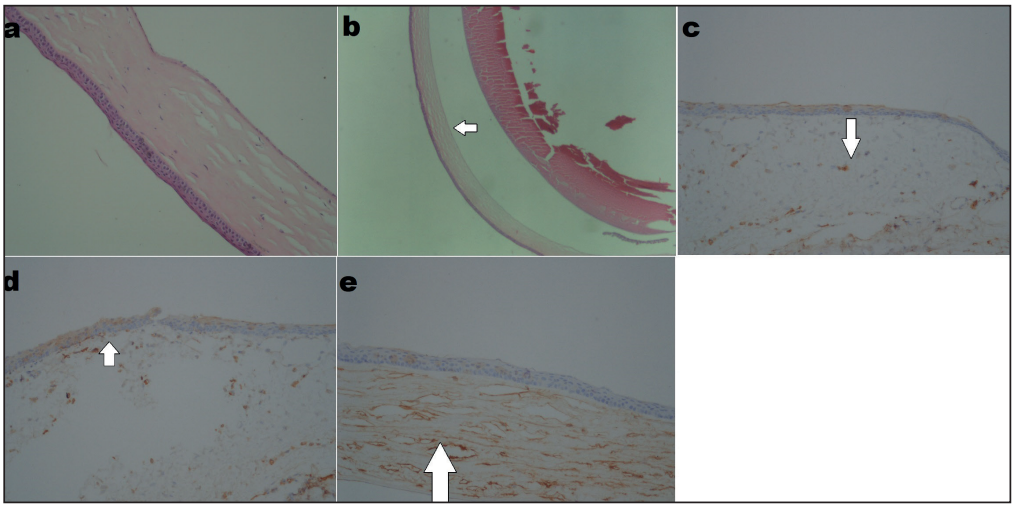

Figure 2. Example corneal samples from the study groups. (**a**) Normal corneal tissue in the control group (H&E x 40); (**b**) Regular corneal tissue (arrow) in treated rats (H&E x 40); (**c**) Corneal stromal 1(+) GFAP staining (arrow) in treated rats (x 100); (**d**) Corneal 1(+) GFAP staining (arrow) in treated rats (x 100); (**e**) High intense 3(+) GFAP staining (arrow) and vascularization in corneal stroma in diabetic rats (x 100).

In this study, we showed that vitamin D treatment either alone or in combination with melatonin was effective on retinal inflammation caused by diabetes. Although combined therapy made a minor difference, nevertheless it was more effective.

Another sight-threatening complication of diabetes is keratopathy manifesting with delayed cornea wound healing, recurrent corneal erosions, and neurotrophic ulcers. However, there is significantly less attention to diabetic keratopathy compared to retinal complications[33]. It has been shown in some studies, that the cornea is more structurally damaged in diabetic rats[34,35]. Similarly, the corneal layers were more irregular and vascularized in diabetic rats in our study. Unfortunately, there were insufficient data collected to statistically quantify the vascularization.

A study conducted by Jing et al[36] showed that calcitriol treatment could significantly suppress the inflammatory markers, such as IL6, TNFA, and IL1B in the rat corneas. Similarly, Saadettin et al[37] demonstrated that 1,25-dihydroxyvitamin D3 inhibits wound healing by decreasing the IL6 level. These studies have focused on the anti-inflammatory aspect of vitamin D treatment.

Another agent with anti-inflammatory and antioxidant properties is melatonin, and its receptors are also present in the cornea[38-41]. However, less information is available about the effects of melatonin on this subject. In a few studies[42,43], melatonin has been shown to affect corneal hydration and corneal wound healing.

In our study, corneal GFAP expression was most pronounced in the untreated diabetic group. When the diabetic rats were treated with vitamin D and melatonin either separately or in combination, GFAP staining decreased dramatically, and more regular corneal layers were observed. Both melatonin and vitamin D can prevent diabetic damage and neuronal injury related to oxidative stress, which may explain the reduction in corneal GFAP with treatment.

The present study has some limitations. Unfortunately, vitamin D and melatonin serum concentrations were not assessed. Also, we have limited data to evaluate vascularization and structural changes. So, further research on vascular and structural parameters would be more fruitful.

Conclusions

To our knowledge, this is the first study that has assessed the effects of vitamin D and melatonin applications when used separately and in combination on eye tissues in STZ-induced diabetes. Our results suggest that vitamin D and melatonin may have therapeutic and protective effects against retinal and corneal damage in diabetic patients. Therefore, this combination could be used as a complementary treatment of diabetes.

Authors' Contribution

Conceptualization: S.B; methodology: S.B, O.N.S., investigation: S.B., E.C., writing-original draft: E.C., S.B., writing-review and editing: S.B., O.C.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1) Zhang Z, Hu X, Qi X, Di G, Zhang Y, Wang Q, Zhou Q. Resolvin D1 promotes corneal epithelial wound healing and restoration of mechanical sensation in diabetic mice. Mol Vis 2018; 24: 274-285.

2) Jiang T, Chang Q, Cai J, Fan J, Zhang X, Xu G. Protective effects of melatonin on retinal ınflammation and oxidative stres in experimental diabetic retinopathy. Oxid Med Cell Longev 2016; 2016: 3528274.

3) Augustin AJ, Keller A, Koch F, Jurklies B, Dick B. Effect of retinal coagulation status on oxidative metabolite and VEGF in 208 patients with proliferative diabetic retinopathy. Klin Monbl Augenheilkd 2001; 218: 89-94.

4) Huang H, Wang Z, Weng SJ, Sun XH, Yang XL. Neuromodulatory role of melatonin in retinal information processing. Prog Retin Eye Res 2013; 32: 64-87.

5) Jiang T, Chang Q, Zhao Z, Yan S, Wang L, Cai J, Xu G. Melatonin-mediated cytoprotection against hyperglycemic injury in Müller cells. PLoS One 2012; 7: e50661.

6) Kaur C, Sivakumar V, Yong Z, Lu J, Foulds WS, and E. A. Ling. Blood-retinal barrier disruption and ultrastructural changes in the hypoxic retina in adult rats: the beneficial effect of melatonin ad-

ministration. The Journal of Pathology 2007; 212: 429-439.

7) Yang LP, Dong YP, Luo WT, Zhu T, Li QW, Zhang LJ, Kong J, Yuan ZW, and Zhao Q. Tissue-Specific Regulatory Effects of Vitamin D and Its Receptor on Calbindin- D28K and Calbindin-D9K. Biochem Mol Biol J 2018; 4: 23.

8) Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol 2010; 2010: 351385.

9) Chang JM, Kuo MC, Kuo HT, Hwang SJ, Tsai JC, Chen HC, Lai YH. 1-alpha,25-Dihydroxyvitamin D3 regulates inducible nitric oxide synthase messenger RNA expression and nitric oxide release in macrophage-like RAW 264.7 cells. J Lab Clin Med 2004;143: 14-22.

10) Mantell DJ, Owens PE, Bundred NJ, Mawer EB, Canfield AE.1 alpha, ,25 Dihydroxyvitamin D (3) inhibits angiogenesis in vitro and in vivo. Circ Res 2000; 87: 214-220.

11) Sprenger CC, Peterson A, Lance R, Ware JL, Drivdahl RH, Plymate SR. Regulation of proliferation of prostate epithelial cells by 1,25-dihydroxyvitamin D3 is accompanied by an increase in insulin-like growth factor binding protein-3. J Endocrinol 2001; 170: 609-618.

12) Bolt MJ, Liu W, Qiao G, Kong J, Zheng W, Krausz T, Cs-Szabo G, Sitrin MD, Li YC. Critical role of vitamin D in sulfate homeostasis: regulation of the sodium-sulfate cotransporter by 1,25-dihydroxyvitamin D3. Am J Physiol Endocrinol Metab 2004; 287: E744-749.

13) Osborne NN, Casson RJ, Wood JP, Chidlow G, Graham M, Melena J. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. Prog Retin Eye Res 2004; 23: 91-147.

14) Yang Z, Wang KK. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci 2015; 38: 364-374.

15) Bignami A, Dahl D. The radial glia of Müller in the rat retina and their response to injury. An immunofluorescence study with antibodies to glial fibrillary acidic (GFA) protein. Exp Eye Res 1979; 28: 63-69.

16) Shan F, Long Y, Qiu W. Autoimmune Glial Fibrillary Acidic Protein Astrocytopathy: A Review of the Literature. Front Immunol 2018; 9: 2802.

17) Rungger-Brändle E, Dosso AA, Leuenberger PM. Glial reactivity, an early feature of diabetic retinopathy. Invest Ophthalmol Vis Sci 2000; 41: 1971-1980.

18) Uchida S, Yokoo S, Yanagi Y, Usui T, Yokota C, Mimura T, Araie M, Yamagami S, Amano S. Sphere formation and expression of neural proteins by human corneal stromal cells in vitro. Invest Ophthalmol Vis Sci 2005; 46: 1620-1625.

19) Zhao G, Chen H, Song Z, Yin H, Xu Y, Chen M. Glial fibrillary acidic protein expression during HSV-1 infection in mouse cornea. APMIS 2014; 122: 128-135.

20) Baydas G, Tuzcu M, Yasar A, Baydas B. Early changes in glial reactivity and lipid peroxidation in diabetic rat retina: effects of melatonin. Acta Diabetol. 2004; 41: 123-128.

21) Özdemir G, Ergün Y, Bakariş S. Melatonin prevents retinal oxidative stress and vascular changes in diabetic rats. Eye 2014; 28:1020-1027.

22) Stene LC, Ulriksen J, Magnus P, Joner G. Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. Diabetologia 2000; 43: 1093-1098.

23) Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R. Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. Diabetologia 1994; 37: 552-558.

24) Shimo N, Yasuda T, Kaneto H, Katakami N, Kuroda A, Sakamoto F, Takahara M, Irie Y, Horikawa K, Miyashita K, Miyatsuka T, Yoshiuchi K, Kawamori D, Sakamoto K, Matsuoka TA, Kosugi K, Shimomura I, Matsuhisa M. Vitamin D deficiency is significantly associated with retinopathy in young Japanese type 1 diabetic patients. Diabetes Res Clin Pract 2014; 106: e41-43.

25) Herrmann M, Sullivan DR, Veillard AS, McCorquodale T, Straub IR, Scott R, Laakso M, Topliss D, Jenkins AJ, Blankenberg S, Burton A, Keech AC; FIELD Study Investigators. Serum 25-hydroxyvitamin D: a predictor of macrovascular and microvascular complications in patients with type 2 diabetes. Diabetes Care 2015; 38: 521-528.

26) Millen AE, Sahli MW, Nie J, LaMonte MJ, Lutsey PL, Klein BE, Mares JA, Meyers KJ, Andrews CA, Klein R. Adequate vitamin D status is associated with the reduced odds of prevalent diabetic retinopathy in African Americans and Caucasians. Cardiovasc Diabetol 2016; 15: 128.

27) Aksoy H, Akçay F, Kurtul N, Baykal O, Avci B. Serum 1,25 dihydroxy vitamin D (1,25(OH)2D3), 25 hydroxy vitamin D (25(OH)D) and parathormone levels in diabetic retinopathy. Clin Biochem 2000; 33: 47-51.

28) Ashinne B, Rajalakshmi R, Anjana RM, Narayan KMV, Jayashri R, Mohan V, Hendrick AM. Association of serum vitamin D levels and diabetic retinopathy in Asian Indians with type 2 diabetes. Diabetes Res Clin Pract 2018 ;139: 308-313.

29) Albert DM, Scheef EA, Wang S, Mehraein F, Darjatmoko SR, Sorenson CM, Sheibani N. Calcitriol is a potent inhibitor of retinal neovascularization. Invest Ophthalmol Vis Sci 2007; 48: 2327-2334.

30) Joergensen C, Hovind P, Schmedes A, Parving HH, Rossing P. Vitamin D levels, microvascular complications, and mortality in type 1 diabetes. Diabetes Care 2011; 34: 1081-1085.

31) Alam U, Amjad Y, Chan AW, Asghar O, Petropoulos IN, Malik RA. Vitamin D Deficiency Is Not Associated with Diabetic Retinopathy or Maculopathy. J Diabetes Res 2016; 2016: 6156217.

32) Patrick PA, Visintainer PF, Shi Q, Weiss IA, Brand DA. Vitamin D and retinopathy in adults with diabetes mellitus. Arch Ophthalmol 2012; 130: 756-760.

33) Lutty GA. Effects of diabetes on the eye. Invest Ophthalmol Vis Sci 2013; 54: 81-87.

34) Bu Y, Shih KC, Kwok SS, Chan YK, Lo AC, Chan TCY, Jhanji V, Tong L. Experimental modeling of cornea wound healing in diabetes: clinical applications and beyond. BMJ Open Diabetes Res Care 2019; 7: e000779.

35) Yamamoto T, Otake H, Hiramatsu N, Yamamoto N, Taga A, Nagai N. A Proteomic Approach for Understanding the Mechanisms of Delayed Corneal Wound Healing in Diabetic Keratopathy Using Diabetic Model Rat. Int J Mol Sci 2018; 19: 3635.

36) Zhang J, Dai Y, Wu D, Xu J. Calcitriol, the Active Metabolite of Vitamin D_3, Inhibits Dry Eye Related Corneal Inflammation In Vivo and In Vitro. Ocul Immunol Inflamm 2019; 27: 257-265.

37) Sel S, Trau S, Paulsen F, Kalinski T, Stangl GI, Nass N. 1,25-dihydroxyvitamin D3 inhibits corneal wound healing in an ex-vivo mouse model. Graefes Arch Clin Exp Ophthalmol 2016; 254: 717-724.

38) Esposito E, Cuzzocrea S. Antiinflammatory activity of melatonin in central nervous system. Curr Neuropharmacol 2010; 8: 228-242.

39) Rada JA, Wiechmann AF. Melatonin receptors in chick ocular tissues: implications for a role of melatonin in ocular growth regulation. Invest Ophthalmol Vis Sci 2006; 47: 25-33.

40) Pintor J, Bautista A, Carracedo G, Peral A. UTP and diadenosine tetraphosphate accelerate wound healing in the rabbit cornea. Ophthalmic Physiol Opt 2004; 24: 186-193.

41) Ostrin LA. Ocular and systemic melatonin and the influence of light exposure. Clin Exp Optom 2019; 102: 99-108.

42) Wahl C, Li T, Takagi Y, Howland H. The effects of light regimes and hormones on corneal growth in vivo and in organ culture. J Anat 2011; 219: 766-775.

43) Crooke A, Guzman-Aranguez A, Mediero A, Alarma-Estrany P, Carracedo G, Pelaez T, Peral A, Pintor J. Effect of melatonin and analogues on corneal wound healing: involvement of Mt2 melatonin receptor. Curr Eye Res 2015; 40: 56-65.