

Corneal endothelial alterations in patients with diabetic macular edema

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Ethics Committee Approval

For this study, approval was obtained from Uludağ University Faculty of Medicine Clinical Research Ethics Committee (decision dated November 21, 2017 and numbered 2017-17 / 11).

All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest

No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Diabetic macular edema (DME) is the main cause of visual loss in diabetic patients. Although it is known that diabetes mellitus could affect all corneal layers, there is no data about the morphological and quantitative changes of corneal endothelium in patients with DME. The aim of this study is to evaluate the corneal endothelial cell density (CD), morphology and central corneal thickness (CCT) in patients with DME.

Methods: This retrospective study included 47 diabetic patients (79 eyes) with DME, 48 diabetic patients (93 eyes) without DME, and 46 nondiabetic subjects (74 eyes). Diagnosis of DME was based on fundoscopy and optical coherence tomography imaging. The corneal endothelial structure and CCT were evaluated using non-contact specular microscopy. The endothelial CD (cells/mm²), coefficient variation of cell area (CV), percentage of hexagonality (HEX) and CCT of the three subgroups were compared.

Results: The mean age of participants was 59.8 (8.3) years. There was no significant difference in terms of age between diabetic patients and control subjects ($P=0.761$). In the diabetic subgroups, HbA1c levels and the number of patients receiving insulin were similar ($P=0.962$, $P=0.082$, respectively), but the mean duration of diabetes was significantly longer in the DME subgroup than in the no-DME subgroup ($P=0.015$). Patients in the DME subgroup did not differ from the patients in the no-DME and control subgroups with regards to endothelial CD and CCT. However, there was a statistically significant decrease in HEX and increase in CV in patients with DME ($P=0.012$, $P=0.012$, respectively).

Conclusion: Patients with DME were found to have higher rates of polymegathism and polymorphism although there were no significant changes in corneal endothelial CD and CCT. These alterations may be the first signs of early corneal damage in patients with DME.

Keywords: Corneal endothelium, Diabetic macular edema, Polymegathism, Polymorphism, Specular microscopy

Introduction

Diabetic macular edema (DME) is the main cause of visual loss in diabetic patients, with an annual incidence of 2.19% [1]. DME can develop in any stage of either nonproliferative or proliferative diabetic retinopathy (DR) [2]. It results in macular thickening due to the failure of the blood-retinal barrier and fluid accumulation within the macula. Although vascular endothelial growth factor (VEGF) overexpression and inflammation are thought to be responsible for DME, the certain pathogenesis is still controversial. In experimental studies, VEGF receptors were expressed on the corneal endothelium [3]. Therefore, the altered expression of growth factors, including VEGF and inflammatory cytokines, may cause corneal endothelial changes in patients with DME [4].

Treatment options for DME include intravitreal injections of anti-VEGF or steroids and photocoagulation [5]. Patients with DME tend to have cataracts because of both chronic hyperglycemia and recurrent intravitreal injections, particularly steroids [6]. Therefore, they are likely to require cataract surgery in the near future. Rarely, pars plana vitrectomy may be required in cases of refractory DME. A healthy corneal endothelium with morphological and functional integrity is necessary for maintaining corneal transparency following intraocular surgeries, particularly after phacoemulsification. It is important to have information about endothelial cells to take intraoperative precautions to minimize the risk of endothelial failure.

Some recent studies have suggested that endothelial cell counts decrease and cell morphology changes in diabetic patients [7-9], while other data have indicated no change in corneal endothelial cell density (CD) [4, 10]. Few studies have examined the correlation between the severity of corneal endothelial changes and DR [11, 12]. However, to the best of the authors' knowledge, no study has evaluated the correlation between corneal endothelial changes and DME. The present study aimed to determine the changes in corneal endothelial CD, endothelial morphology and central corneal thickness (CCT) in patients with DME.

Materials and methods

The medical reports of patients with type II DM who attended the ophthalmology outpatient clinic between January 2016 and November 2017 were identified retrospectively. The diagnosis of type II DM was based on the patients' medical history. Demographic characteristics, most recent HbA1c levels, type of antidiabetic medication and duration of DM were recorded from the medical data. Diabetic patients with no or mild DR were included in the study. Patients with proliferative DR, glaucoma, corneal diseases and a history of previous intraocular surgery, intravitreal injection, argon laser photocoagulation and contact lens-wearing were excluded from the study. Since the corneal endothelium is affected by age, care was taken to ensure that the ages of the patients in the study groups were similar.

Diabetic patients were divided into two subgroups based on the presence of DME. The presence of DME and stages of DR were determined via a dilated fundus examination, optical coherence tomography (Optovue, iVue, USA) and fluorescein

angiography. Patients with center-involving DME, which was defined as fovea-involving fluid on optical coherence tomography in association with the clinical diagnosis of diabetic maculopathy [13], were included in the DME subgroup. Diabetic patients with normal foveal configuration without any fluid on optical coherence tomography were included in the no-DME subgroup. The control group consisted of age-matched nondiabetic subjects who were admitted to the ophthalmology clinic for a routine examination. All subjects underwent a complete ophthalmologic examination, which included testing for best corrected visual acuity, slit lamp examination, intraocular pressure measurement with pneumotonometer and funduscopy. This study was approved by the Ethics Committee of the Uludag University Faculty of Medicine on 21.11.2017 with the number of 2017-17/11 and conducted per the Declaration of Helsinki.

Corneal endothelial CD (cells/mm²), coefficient variation of cell area (CV), the percentage of hexagonal cells (HEX) and CCT were analyzed automatically using a non-contact specular microscopy (NSP-9900, NonconRobo, Konan, Japan). Pictures were taken from the central cornea in a suitable head position while the patient was sitting. The measurements were obtained three times, and the mean was calculated.

The demographic characteristics, duration of DM, HbA1c levels, central macular thickness, corneal endothelial parameters, CCT and intraocular pressure of the three subgroups were then compared.

Statistical analysis

The Shapiro–Wilk test was used to decide the distribution of the data. The results were presented as mean (\pm standard deviation) or frequency and percentage. According to pilot study, using pooled standard deviation of CV (5.92), a power analysis indicated that a total sample of 46 people would be needed to detect effect size ($d=0.27$) with 80% power with alpha at 0.05, 2-sided significance level. Normally distributed data were compared with an independent sample *t*-test or one-way ANOVA. Kruskal–Wallis and Mann–Whitney U tests were used for non-normally distributed data. The Bonferroni test was used as a multiple comparison test. Categorical variables were compared using Pearson's chi-square test and Fisher's exact test between groups. A *P*-value of <0.05 was considered the significance level. Statistical analyses were performed with IBM SPSS ver.23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).

Results

A total of 95 diabetic patients and 46 healthy subjects enrolled in the present study. The mean age was 59.8 (8.3) years. Seventy-five patients (53.2%) were female. Seventy-nine eyes of 47 diabetic patients had DME with a mean central macular thickness of 417.7 (115.7) μ . These were classified as the DME subgroup. Ninety-three eyes of 48 diabetic patients without DME were classified as the no-DME subgroup. Seventy-four eyes of 46 nondiabetic subjects were classified as a control group. There were no statistically significant differences between the three groups in terms of age and sex (Table 1).

In the diabetic subgroups, no patient had proliferative DR. The mean duration of DM was 12.6 (6.4) years, and the

mean HbA1c level was 8.8 (2.2) %. There were no differences between the diabetic groups in terms of HbA1c levels ($P=0.962$) and frequency of insulin usage ($P=0.082$), but the mean duration of DM was significantly longer in the DME subgroup than in the no-DME subgroup ($P=0.015$) (Table 1).

The endothelial CD was 2641.4 cells/mm² in the DME subgroup, 2648.6 cells/mm² in the no-DME subgroup, and 2690.6 cells/mm² in the control group ($P=0.429$) (Table 2). In the DME subgroup, the mean HEX was 43.3 (5.9), and it was significantly lower than the no-DME and control subgroups ($P=0.012$). Similarly, the mean CV was 45.1 (6.8) in the DME subgroup, which was significantly higher than in the no-DME and control subgroups ($P=0.012$). There were no significant differences between the three subgroups in terms of CCT and intraocular pressure ($P=0.188$, $P=0.076$, respectively) (Table 2).

Table 1: Demographic characteristics and clinical properties of patients

Parameters	DME group n=47 pts	No-DME group n=48 pts	Control group n=46 pts	P-value
Gender (F/M)	23 F / 24 M	27 F / 21 M	25 F / 21 M	0.761
Age in years, mean (SD)	61.32 (6.9)	59.54 (8.8)	58.5 (9.1)	0.242
Duration of DM, years, mean (SD)	14.2 (6.3)	11.0 (6.1)	-	0.015
HbA1c level, mean (SD)	8.8 (1.9)	8.8 (2.6)	-	0.962
Insulin usage (%)	35 (74.5)	27 (57.4)	-	0.082

DME: diabetic macular edema, DM: diabetes mellitus, pts: patients, F: female, M: male, SD: standard deviation

Table 2: Central macular thickness, corneal parameters and intraocular pressure of study eyes

Parameters	DME group n=79 eyes	No-DME group n=93 eyes	Control group n=74 eyes	P-value
CMT	417.7 (115.7)	252.0 (19.5)	256.0 (27.0)	<0.001
CD	2641.4 (238.0)	2648.6 (261.1)	2690.6 (256.6)	0.429
CV	45.1 (6.8)	42.2 (6.4)	42.6 (6.7)	0.012
HEX	43.3 (5.9)	46.3 (7.1)	45.6 (8.1)	0.012
CCT	541.8 (35.5)	551.0 (35.7)	549.5 (31.7)	0.188
IOP	17.7 (2.9)	18.0 (2.8)	17.0 (2.3)	0.076

Parameters are written as mean (standard deviation). DME: diabetic macular edema, CMT: central macular thickness, CD: cell density, CV: coefficient of variation, HEX: hexagonality, CCT: central corneal thickness, IOP: intraocular pressure

Discussion

Diabetes mellitus can affect all corneal layers, and several corneal disorders are present in more than 70% of diabetic patients [14]. It can also damage the corneal endothelium, which is the layer responsible for maintaining corneal transparency. However, data about the correlation between corneal endothelial changes and the severity of DR is limited. While most studies on this topic have found that the stage of DR is not correlated with corneal endothelial findings [9, 15, 16], only a few studies have reported that the number of endothelial cells is significantly lower in eyes with proliferative DR [11, 14, 17].

Diabetic macular edema can be considered a separate entity from DR because it occurs in isolation without other signs of microangiopathy in the peripheral retina [5]. In addition, it can accompany any stage of DR. To the best of our knowledge, no study has evaluated the morphological and quantitative changes of corneal endothelium in patients with DME.

The endothelial cells of retinal capillaries are joined to each other by tight junctions occurring the inner blood-retinal barrier to regulate retinal fluid level. In diabetes mellitus (DM), Muller cells and retinal endothelial cells produce several chemokines, such as VEGF, tumor necrosis factor α , interleukin 1 β and matrix metalloproteinase, due to chronic inflammation and hyperglycemia. These inflammatory cytokines cause the tight junctions to break down, which, in turn, causes DME. Corneal endothelial cells also have tight junctions between them

to maintain corneal dehydration and clarity. With a mechanism similar to retinal pathology, corneal endothelial junctions may break down, and paracellular permeability may increase through junctional alterations in eyes with DME [2, 18].

In the present study, the mean endothelial CD was 2641.4 cells/mm² in the DME subgroup. This was similar to the no-DME and control subgroups in this study. In the last 10 years, numerous studies have investigated endothelial CD alterations in diabetic patients by comparing them with healthy controls. Most of these studies have reported a decrease in the endothelial CD in diabetic patients, particularly in those with type I DM [19, 24]. However, similar to the present study, some studies did not find statistically significant differences in endothelial CD between diabetics and healthy controls. All these studies included patients with type II DM, as did our sample [10, 19, 20]. The reason that CD was more affected in type I than type II DM may be due to the younger onset and longer duration of disease in type I DM. In addition, in most studies, the duration of DM was correlated with a decrease in endothelial CD in diabetic patients [21, 22, 25].

Corneal endothelial cells have hexagonal shapes, usually with a mosaic pattern, and do not proliferate in vivo. Physiologically, the number of endothelial cells decreases with ageing, but ocular trauma and surgery cause endothelial cell loss more than usual. In this instance, the defective area is compensated via enlargement of residual cells. These alterations can be determined by specular microscopy as polymegathism (variability in cell size) and polymorphism (variability in cell shape) [26].

In the present study, patients with DME had higher rates of polymegathism [45.1 (6.8)] and polymorphism [43.3 (5.9)] than the no-DME and control subgroups. In most previous studies, polymegathism and polymorphism accompanied lower endothelial CD in diabetic patients compared to healthy individuals [22, 25, 27]. However, polymegathism and polymorphism could be a more sensitive precursor of endothelium under stress before the occurrence of a significant decrease in CD [28]. This may be because the endothelial CD will decrease by 1% if only one cell is lost in a cluster of 100 cells, which would be an insignificant decrease. However, if a six-sided cell is lost in the cluster of 100 cells, two (2%) or six (6%) adjacent cells will show significant morphological changes for repairing the defect [26]. Therefore, higher rates of polymegathism and polymorphism in eyes with DME may be explained as early diabetic corneal endothelial damage before significant changes in endothelial CD. Similarly, in a recent study, Leelawongtawun et al. [19] found that when diabetes progresses, hexagonal cells decreased at first (in > 1-year diabetics). This was followed by polymegathism (in > 2 years diabetics) before changes in endothelial CD occurred. However, higher rates of polymegathism and polymorphism could also be associated with diabetes duration, which was longer in patients with DME than in those without DME in the present study [14.2 (6.3) and 11.0 (6.1) years, respectively].

Another indicator of endothelial cell dysfunction is CCT. Endothelial cell Na/K ATPase and tight junctions are responsible for controlling the entrance of aqueous humour into the corneal stroma to maintain corneal dehydration. If endothelial cell loss occurs, the frequency of tight junctions

between cells decreases, allowing more aqueous humour to enter the corneal stroma. Consequently, CCT increases with the loss of corneal transparency [29]. Regarding CCT in the present study, there was no significant difference between subjects in the DME, no-DME, and control subgroups. This could be due to the similar density of endothelial cells in the three subgroups. This outcome was in concordance with those of El-Agamy et al. [9], and Choo et al. [16], while other authors have reported higher CCT in those with type 2 DM compared to nondiabetics [7, 10].

Only a few studies have compared the corneal endothelial changes that occur in diabetics and nondiabetics after cataract surgery. Recently, Sahu et al. [30] found that diabetic patients showed a significantly higher loss of corneal endothelial density after phacoemulsification than nondiabetic patients with similar nuclear grading and phaco energy used. They suggested that a more careful approach during phacoemulsification was required in diabetic patients, even in the presence of good glycemic control. It was, therefore, important to have information about the corneal endothelial integrity of diabetic patients prior to cataract surgery to reduce the risk of endothelial decompensation.

The strength of the present study was that patients were age-matched among three subgroups to eliminate possible age-related bias in corneal endothelium. No patient had a history of intraocular surgery, intravitreal injection or laser photocoagulation in order to avoid influencing the corneal endothelial parameters. Diabetic patients with and without DME had similar glycemic status regarding HbA1c levels. No patient had proliferative DR that could have had a probable effect on corneal endothelium and CCT, as suggested by several studies [12, 13].

Limitations

Limitations of the study include retrospective data collection, lack of multivariate study and different lengths of diabetes duration in patients with and without DME. Future prospective studies are required for confirming the corneal alterations in patients with DME.

Conclusion

This study suggested higher rates of polymegathism and polymorphism in patients with DME, although there were no significant changes in endothelial CD and CCT. These alterations may be the first signs of early corneal damage. Therefore, evaluation of corneal endothelium in patients with DME should be a part of routine examination prior to intraocular surgery. Extra care should be taken when treating patients with low endothelial reserve.

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