Impact of Diabetes Mellitus Type 2 on Donor Corneas Endothelial Cell Density

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ABSTRACT

Introduction: Diabetic eye disease accounts for many ocular manifestations other than diabetic retinopathy, some affecting the ocular surface and anterior segment.

Objectives: to compare the endothelial cell density (ECD) of donor corneas from diabetic vs. non-diabetic patients.

Methods: Retrospective study of donor corneas from a tertiary care center. Data screening and extraction were performed from the hospital’s Eye Bank database, specular microscopy and clinical records from emergency department and outpatient clinic. All consecutive donor corneas were included, given specular microscopy had quality readings and data records were complete. Donors were divided in two groups (diabetic and non-diabetic), and specular microscopy data compared. Primary outcome: ECD. Secondary outcomes: standard deviation (SD), coefficient of variation (CV), percentage of hexagonal cells (HEX), average cell area (AVE) and overall graft success (absence of graft failure and rejection).

Results: 73 corneas were included, from 42 donors (39.7% females). Mean donor age was 56 ± 11 years. Fifteen patients were diabetic (20.5%). Mean ECD in diabetics was 2523.67 ± 299.27 cells/mm2, vs. 2528.28 ± 368.92 cells/mm2 in non-diabetics (p=0.965). Mean SD was 126.05 ± 42.37 in diabetics vs. 134.6 ± 50.12 in non-diabetics (p=0.53). CV was 32.93 in diabetic patients, and 31.23 in non-diabetic (p=0.51). HEX was comparable in both groups, with 49.73% in diabetics and 51.04% in non-diabetics (p=0.086). Overall graft success rates were comparable between groups.

Conclusions: No significant endothelial differences were found between diabetic and non-diabetic groups.

Keywords: Corneal Endothelium; Corneal Transplantation; Diabetes Mellitus; Hyperglycemia

Diabetes mellitus type 2 (DM2) is a chronic epidemic disorder with an estimated global prevalence among adults of 8.5%1; It is projected that DM2 will be the seventh leading cause of death in the world by 20301. The high mortality and morbidity rates make it increasingly common among organ and tissue donors – the impact of which is often still unclear.

The main pathological mechanisms of diabetic end-organ disease are through non-enzymatic glycosylation, generating advanced glycation end products (AGEs). AGEs lead to altered protein structure and function; generate reactive oxygen species (ROS); and covalently crosslink to nearby proteins as collagen. Combined, these pathways lead to macro- and microvascular induced multiple organ dysfunction.2-4

Diabetic ocular pathology has been mostly linked to diabetic retinopathy, though other associations have been described in scientific literature. There also seems to be a group of diabetic induced corneal changes, globally designated as diabetic corneal disease. It manifests through the decreased innervation at the subbasal plexus (a way of peripheral neuropathy), which in turn induces a neurotrophic (diabetic) keratopathy with reduced epithelial cell adhesion, abnormal wound repair, recurrent erosions and corneal edema caused by altered barrier function.5-8 Diabetic corneal disease may also account for the thickening of the stroma and basement membranes by collagen crosslinking, increase in global corneal stiffness, decreased endothelial cell density, and endothelial pleomorphism.5-8

Several mechanisms have been proposed to explain diabetic induced endothelial disease: (1) activation of apoptotic pathways and collagen crosslinking by AGEs,5-8,11 (2) reduced activity of Na+-K+ ATPase, inducing morphology and permeability changes,12-13 and (3) altered aqueous humor composition, leading to posterior corneal dysfunction.14,15 Even though the pathological pathways of diabetic corneal disease are far from enlightening, these changes seem to affect up to 70% of diabetic patients—yet they are frequently underdiagnosed.5,7,16-18

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The purpose of our study was to assess the impact of DM2 on donor corneas endothelial cell density.

**MATERIALS AND METHODS**

We performed a retrospective analysis of donor corneas harvested in a tertiary care center (Hospital de Santa Maria, Lisboa, Portugal), from January 2016 to September 2017. The Institutional Review Board of the Lisbon Academic Medical Center agreed with the conduct of this study. Eligible donors met national standards for human tissue transplantation and included both donors after cardiac death and multi-organ and tissue donors (after brain death confirmed). All corneas included in this analysis were collected by medical doctors, stored in EUSOL-C® (Alchimia, Padova, Italy) and assessed by experienced dedicated corneal ophthalmologists at the local Eye Bank, through slit lamp examination and non-contact specular microscopy (CellCheck D+®; Konan Medical, Irvine, CA). Data screening and extraction were performed from the databases of the local Eye Bank and specular microscopy readings. In addition, we screened donors’ data records from the outpatient clinic and emergency department, to crosscheck for medical history of DM2.

All consecutive corneas were included in this study, provided specular microscopy had quality readings and medical records were complete. Donors were classified as diabetic whenever data screening retrieved documented past medical history of DM2, home insulin use or diabetic end-organ damage with explicit causal relation (diabetic retinopathy or macular edema, nephropathy, polyneuropathy, or peripheral vascular disease); if data screening failed to detect the aforementioned history, donors were otherwise classified as non-diabetic. Specular microscopy data were then compared for both study groups.

We opted not to screen for further information on diabetic donors’ comorbidities or metabolic control, such as last glycated hemoglobin (A1c) levels, pharmacological treatment with oral antidiabetic drugs and/or insulin, and duration of disease, as these were expected to be unequally documented in the assessed records. For this reason, no distinction was made between diabetic patients with advanced and non-advanced disease, nor performed any type of disease severity grading.

Primary outcome for this analysis was the endothelial cell density (ECD). Secondary outcomes were: standard deviation (SD), coefficient of variation (CV), percentage of hexagonal cells (HEX), average cell area (AVE) and overall graft success (absence of graft failure and rejection).

Statistical analysis was performed with SPSS software® (IBM, New York, USA). Chi-squared test was used for purposes of comparing dichotomous outcomes, while two-tailed unpaired student t-test was employed for the comparisons of continuous outcomes, at the significance level $\alpha$ of 0.05.

**RESULTS**

**Baseline demographic data**

Seventy-three corneas were included in this analysis, from a total of 46 donors. Overall, the majority of the included corneas came from male donors (n=42 corneas, 59.2%), and thirty-six corneas were from donors’ right eyes (49.3%). Mean donor age was of 55.6 ± 11.5 years old, ranging from 24 to 71 years old. The main causes of death per included cornea were terminal cancer (n=23, 31.5%), cerebrovascular accident (n=19, 26%) and acute coronary syndrome (n=10, 13.7%), together comprising more than two thirds of the included sample. Fifteen donors were classified as diabetic (20.5%) according to the aforementioned criteria, with the remaining 58 donors (79.5%) categorized as non-diabetic.

Diabetic and non-diabetic groups were balanced regarding age (p=0.22), gender (p=0.21), most prevalent causes of death (p=0.07), and laterality (right vs. left; p=0.77). Table 1 depicts baseline demographic data for both diabetic and non-diabetic donor groups.

**OUTCOMES**

Mean ECD was 2523.67 ± 299.27 cells/mm$^2$ in the diabetic pool of donors, compared to 2528.28 ± 368.92 cells/mm$^2$ among non-diabetics (p=0.97). Average SD was 126.05 ± 42.37 in diabetic patients, and 134.6 ± 50.12 in non-diabetic (p=0.51). Diabetic donors had a mean CV of 32.93 vs. 31.23 in non-diabetic donors (p=0.53). HEX was comparable in both groups as well, with 49.73% in diabetics and 51.04% in non-diabetics (p=0.79). Mean AVE in diabetic donors was of 401.33μm$^2$ vs. 404.51μm$^2$ in non-diabetic donors (p=0.86). Table 2 illustrates the relative mean differences in endothelial outcomes of diabetic patients, comparing to non-diabetics.

From the corneas included in this analysis, seven were excluded from transplantation for endothelial disease as low endothelial cell density and Fuchs’ endothelial dystrophy; two of these corneas were from diabetic donors (13.2%) and five from non-diabetic (9.4%; p-value of comparison=0.8). Additionally, eight corneas were excluded from transplantation for positive screening of infectious disease
# Impact of Diabetes Mellitus Type 2

## Table 1. Baseline demographic data

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age, years (mean ± SD)</th>
<th>Male, n (%)</th>
<th>Right eye, n (%)</th>
<th>Main causes of death, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>15</td>
<td>59.1 ± 10.8</td>
<td>11 (73.3%)</td>
<td>8 (53.3%)</td>
<td>CVA: 5 (33%)</td>
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<td>ACS: 4 (26.7%)</td>
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<td></td>
<td>Cancer: 3 (20%)</td>
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<tr>
<td>Non-diabetic</td>
<td>58</td>
<td>55.1 ± 11.2</td>
<td>31 (53.4%)</td>
<td>28 (48.3%)</td>
<td>Cancer: 20 (34.5%)</td>
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<td></td>
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<td></td>
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<td></td>
<td>CVA: 14 (24.1%)</td>
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<td></td>
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<td>ACS: 6 (10.3%)</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndrome; CVA, cerebrovascular accident; SD, standard deviation.

## Table 2. Summary of endothelial outcomes

<table>
<thead>
<tr>
<th></th>
<th>ECD, cells/mm² (mean ± SD)</th>
<th>SD (mean ± SD)</th>
<th>CV (mean)</th>
<th>HEX, % (mean)</th>
<th>AVE, μm² (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>2523.67 ± 299.27</td>
<td>126.05 ± 42.37</td>
<td>32.93</td>
<td>49.73</td>
<td>401.33</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>2528.28 ± 368.92</td>
<td>134.6 ± 50.12</td>
<td>31.23</td>
<td>51.04</td>
<td>404.51</td>
</tr>
</tbody>
</table>

Mean difference (diabetic – non-diabetic), %

-0.18 -6.78 5.16 -2.63 -0.79

P-value of comparison

0.97 0.51 0.53 0.79 0.86

AVE, average cell area; CV, coefficient of variation; ECD, endothelial cell density; HEX, percentage of hexagonal cells; SD, standard deviation.

## Table 3. Characteristics of the transplanted patients

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age, years (mean ± SD)</th>
<th>Male, n (%)</th>
<th>Right eye, n (%)</th>
<th>Surgical indication, n (%)</th>
<th>Keratoplasty procedures</th>
<th>Follow-up, months (mean ± SD)</th>
<th>Transplant outcome, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With diabetic donor</td>
<td>11</td>
<td>61.4 ± 23.5</td>
<td>7 (63.6)</td>
<td>6 (54.5)</td>
<td>BK: 3 (27.3)</td>
<td>DALK: 2 (18.2)</td>
<td>16.6 ± 6.7</td>
<td>Overall graft success: 9 (81.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FED: 1 (9.1)</td>
<td>DMEK: 1 (9.1)</td>
<td></td>
<td>“High risk” graft failure: 2 (18.2)</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>KC: 3 (27.3)</td>
<td>PK: 8 (72.7)</td>
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<td></td>
<td></td>
<td>Corneal opacity: 2 (18.2)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>PPD: 1 (9.1)</td>
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<td></td>
<td></td>
<td></td>
<td>Tectonic: 1 (9.1)</td>
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<tr>
<td>With non-diabetic donor</td>
<td>48</td>
<td>46.4 ± 21.9</td>
<td>25 (55.6)</td>
<td>24 (53.3)</td>
<td>BK: 5 (10.4)</td>
<td>DALK: 12 (25)</td>
<td>17.3 ± 6.3</td>
<td>Overall graft success: 39 (81.3)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FED: 6 (12.5)</td>
<td>DMEK: 3 (6.3)</td>
<td></td>
<td>“High risk” graft failure: 2 (4.17)</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td>Corneal opacity: 10 (20.8)</td>
<td>PK: 33 (68.8)</td>
<td></td>
<td>Endothelial rejection: 7 (14.6)</td>
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<tr>
<td></td>
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<td>KC: 16 (33.3)</td>
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<td>Peter’s anomaly: 1 (2.1)</td>
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<td>PRSE: 1 (2.1)</td>
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<td>Salzmann: 1 (2.1)</td>
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<td>Tectonic: 7 (14.6)</td>
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<td>Therapeutic: 1 (2.1)</td>
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</tbody>
</table>

P-value of comparison

-0.05 0.63 0.94 - - 0.75 0.97

ABK, bullous keratopathy; DALK, deep anterior lamellar keratoplasty; DMEK, Descemet membrane endothelial keratoplasty; FED, Fuchs endothelial dystrophy; KC, keratoconus; PK, penetrant keratoplasty; PPD, posterior polymorphous dystrophy; PRSE, post refractive surgery ectasia; SD, standard deviation.
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Our study mainly assessed the impact of DM2 on donor corneas’ endothelial cell density, including a total of 73 corneas from 46 donors. Our findings denied the impact of diabetes status on endothelial global health, either specular microscopy parameters or clinical graft success rates.

Other papers have been published on the effects of DM2 on human corneas, though there are still contradictions on this matter. Several papers, both on living and deceased patients, have reported results similar to ours, denying the effect of the metabolic status on the endothelial health;6,23-31 on the other hand, some studies did report lower ECD in the corneas of diabetic patients.31-36 Some methodological issues may explain these differences, and must be acknowledge. One, as in our study, is the lack of severity grading and insulin status assessment, preventing the ability to detect differences concerning severe forms of disease. In instance, Liaboe and colleagues37 found differences only between insulin-dependent diabetics with history of medical complications, compared to non-insulin dependent diabetics and non-diabetics, suggesting only severe diabetes may significantly impact the health of corneal endothelium. However, the authors stated these corneas were still equally likely to be included in the donor pool for transplantation, rejecting the clinical significance of the endothelial findings.

The other main issue that might have impacted our results is the lack of power to detect small differences, granted by a large sample size. Nonetheless a large study on this topic, published by Margo et al;26 included total of 27,948 donor eyes from 14,532 donors; this high-powered sample still failed to find differences in ECD according to disease status.

Finally, the main issue regarding our and other analyses is the retrospective character, extracting data from the donors’ medical records. As previously stated, this design cannot exclude bias by misclassification of disease status. However, prospective assessment of disease status among future cornea donors would be unpractical and lingering for obvious reasons.

Our study did not find differences regarding overall graft success, and this seems to be a less controversial matter.38 We did not assess endothelial graft preparation technical difficulty; however, current literature describes a greater adhesion strength of the Descemet membrane in diabetic corneas,39 maybe related to some reports of endothelial graft preparation challenges in corneas from diabetic donors.24,27,40

In conclusion, our results support the raising evidence of diabetic corneas suitability for transplant. Methodological issues regarding our and other analysis are difficult to control, and raise awareness to the importance of robust data collection in our daily practice.

LITERATURE CITED

Impact of Diabetes Mellitus Type 2


