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-diabetic (p=0.51). CV was 32.93 in diabetic patients, and 31.23 in non-diabetic (p0.53). HEX was comparable in both groups, with 49.73% in diabetics and 51.04% in non-diabetics (p=079). Diabetic donors had an AVE of 401.33µm2 vs. 404.51µm2 in non-diabetic donors (p=0.086). Overall graft success rates were comparable between groups.

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

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 2030[1]. 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;[3,8-11] (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,[19-21] 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 unevenly 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).[22] 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 α 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

Table 1. *Baseline demographic data*

	N	Age, years (mean ± SD)	Male, n (%)	Right eye, n (%)	Main causes of death, n (%)
Diabetic	15	59.1 ± 10.8	11 (73.3%)	8 (53.3%)	CVA: 5 (33%) ACS: 4 (26.7%) Cancer: 3 (20%)
Non-diabetic	58	55.1 ± 11.2	31 (53.4%)	28 (48.3%)	Cancer: 20 (34.5%) CVA: 14 (24.1%) ACS: 6 (10.3%)
P-value of comparison	-	0.22	0.21	0.77	0.07

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

Table 2. *Summary of endothelial outcomes*

	ECD, cells/mm^2 (mean ± SD)	SD (mean ± SD)	CV (mean)	HEX, % (mean)	AVE, □m^2 (mean)
Diabetic	2523.67 ± 299.27	126.05 ± 42.37	32.93	49.73	401.33
Non-diabetic	2528.28 ± 368.92	134.6 ± 50.12	31.23	51.04	404.51
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*

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

(n=5, 2 from diabetic patients) and cancelled surgery for lack of anesthetic conditions (n=2).

Overall grafted patients presented similar characteristics across groups, but patients receiving corneas from non-diabetic donors were significantly younger (46.4 vs. 61.4 years old, p=0.051). Main indications for surgery were keratoconus (n=19), post-trauma or –infection corneal opacity (n=12), bullous keratopathy (n=8) and Fuchs endothelial dystrophy (n=7). In average, patients were followed for 17 ± 6.3 months post-surgery, ranging from 8 to 28 months (diabetic donors: 16.6 ± 6.7 months, n=11; non-diabetic donors: 17.3 ±6.3 months, n=48; p-value of comparison: 0.75). The rates of overall graft success (absence of graft failure and rejection) were comparable between groups: diabetics 81.8% vs non-diabetics 81.3% (p=0.97). Table 3 depicts the main characteristics of the transplanted patients.

DISCUSSION

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 colleagues[37] 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 a;[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

1. Mathers CD, Loncar D (2006) Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 3(11): e442. DOI: 10.1371/journal.pmed.0030442
2. Stitt AW. Advanced glycation: an important pathological event in diabetic and age related ocular disease. *Br J Ophthalmol* 2001;85:746–753. DOI: 10.1136/bjo.85.6.746
3. Kaji Y, Usui T, Oshika T, et al. Advanced glycation end products in diabetic corneas. *Invest Ophthalmol Vis Sci* 2000;41:362–368. 17. URL: http://iovs.arvojournals.org/article.aspx?articleid=2199847
4. Sady C, Khosrof S, Nagaraj R. Advanced Maillard reaction and crosslinking of corneal collagen in diabetes. Biochem Biophys Res Commun 1995;214:793–797. DOI: 10.1006/bbrc.1995.2356
5. Schultz RO, Van Horn DL, Peters MA, et al. Diabetic keratopathy. Trans Am Ophthalmol Soc 1981;79:180–99. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1312182/?page=1
6. Quadrado MJ, Popper M, Morgado AM, et al. Diabetes and corneal cell densities in humans by in vivo confocal microscopy. *Cornea* 2006; 25(7):761-8. DOI: 10.1097/01.ico.0000224635.49439.d1
7. Vieira-Potter VJ, Karamichos D, Lee DJ. Ocular complications of diabetes and therapeutic approaches. *BioMed Research International* 2016. DOI: 10.1155/2016/3801570
8. Bikbova G, Oshitari T, Tawada A, et al. Corneal changes in diabetes mellitus. Current Diabetes Reviews 2012; 8:294-302. DOI: 10.2174/157339912800840479
9. Malik NS, Moss SJ, Ahmed N, et al. Ageing of the human corneal stroma: structural and biochemical changes. Biochim Biophys Acta 1992;1138:222–8. DOI: 10.1016/0925-4439(92)90041-K
10. Yucel I, Yucel G, Akar Y, et al. Transmission electron microscopy and autofluorescence findings in the cornea of diabetic rats treated with aminoguanidine. *Can J Ophthalmol* 2006; 41: 60-6. DOI: 10.1139/i05-105
11. Kaji Y, Amano S, Usui T, et al. Expression and function of receptors for advanced glycation end products in bovine corneal endothelial cells. *Invest Ophthalmol Vis Sci* 2003; 44:521-8. DOI: 10.1167/iovs.02-0268

12. Herse PR. Corneal hydration control in normal and alloxan-induced diabetic rabbits. I*nvest Ophthalmol Vis Sci* 1990; 31:2205-13. URL: http://iovs.arvojournals.org/article.aspx?articleid=2160377
13. Weston B, Bourne W, Polse K, et al. Corneal hydration control in diabetes mellitus. I*nvest Ophthalmol Vis Sci* 1995; 36:586-95. URL: http://iovs.arvojournals.org/article.aspx?articleid=2179865
14. Marano CW, Matschinsky FM. Biochemical manifestations of diabetes mellitus in microscopic layers of the cornea and retina. *Diabetes Metab Re*v 1989; 5:1-15. DOI: 10.1002/dmr.5610050102
15. Chiang SY, Tsai ML, Wang CY, et al. Proteomic analysis and identification of aqueous humor proteins with a pathophysiological role in diabetic retinopathy. *Journal of Proteomics* 2012; 75:2950-9. DOI: 10.1016/j.jprot.2011.12.006
16. Herse PR. A review of manifestations of diabetes mellitus in the anterior eye and cornea. *American Journal of Optometry and Physiological Optics* 1988; 65(3):224-30. DOI: 10.1097/00006324-198803000-00013
17. Skarbez K, Priestley Y, Hoepf M et al. Comprehensive review of the effects of diabetes on ocular health. *Expert Review of Ophthalmology* 2010; 5(4):557-77. DOI: 10.1586/eop.10.44
18. Owen CG, Newsom RSB, Rudnicka AR et al. Vascular response of the bulbar conjunctiva to diabetes and elevated blood pressure. *Ophthalmology* 2005; 112(10):1801-8. DOI: 10.1016/j.ophtha.2005.04.030
19. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. *Official Journal of the European Union* 2004; L102/48. Accessed in April 1st 2018, at http://eur-lex.europa.eu/Lex-UriServ/LexUriServ.do?uri=OJ:L:2004:102:0048:0058:en:PDF.
20. Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells. *Official Journal of the European Union* 2006; L38/40. Accessed in April 1st 2018, at https://hpscreg.eu/docs/downloads/l_03820060209en00400052.pdf.
21. Decreto Lei n. 12/2009 de 26 de Março da Assembleia da República. Diário da República: I série, n. 60 (2009). Accessed in April 1st 2018, at https://dre.pt/application/conteudo/603264.
22. IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.
23. Schultz R, Matsuda M, Yee RW et al. Corneal endothelial changes in type I and type II diabetes mellitus. *Am J Ophthalmol* 1984; 98(4):401-10. DOI: 10.1016/0002-9394(84)90120-X
24. Price MO, Lisek M, Feng MT, et al. Effect of donor and recipient diabetes status on Descemet membrane endothelial keratoplasty adherence and survival. *Cornea* 2017; 36(10):1184-8. DOI: 10.1097/ICO.0000000000001305
25. Lass JH, Riddlesworth TD, Gal RL, et al. The effect of donor diabetes history on graft failure and endothelial cell density 10 years after penetrating keratoplasty. *Ophthalmology* 2015;122(3):448-56. DOI: 10.1016/j.ophtha.2014.09.012
26. Margo JA, Munir WM, Brown C, et al. Association between endothelial cell density and transplant suitability of corneal tissue with type 1 and type 2 diabetes. *JAMA Ophthalmo*l 2016. DOI: 10.1001/jamaophthalmol.2016.5095
27. Greiner MA, Rixen JJ, Wagoner MD, et al. Diabetes mellitus increases risk of unsuccessful graft preparation in Descemet membrane endothelial keratoplasty: A Multicenter Study. *Cornea* 2014; 33. DOI: 10.1097/ICO.0000000000000262
28. Módis L, Szalai E, Kertész K, et al. Evaluation of the corneal endothelium in patients with diabetes mellitus type I and II. *Histol Histopathol* 2010; 25(12):1531-7. DOI: 10.14670/HH-25.1531
29. Larsson L, Bourne W, Pach J, et al. Structural and function of the corneal endothelium in diabetes mellitus type I and II. *Arch Ophthalmol* 1996; 114:9-14.7
30. Matsuda M, Ohguro N, Ishimoto I, et al. Relationship of corneal endothelial morphology to diabetic retinopathy, duration of diabetes and glycemic control. *Jpn J Ophthalmol* 1990; 34:53-6. URL: https://www.ncbi.nlm.nih.gov/pubmed/2362374
31. El-Agamy A, Alsubaie S. Corneal endothelium and central corneal thickness changes in type 2 diabetes mellitus. *Clin Ophthalmol* 2017; 11:481-6. DOI: 10.2147/OPTH.S126217
32. Szalai E, Deák E, Módis L, et al. Early corneal cellular and nerve fiber pathology in young patients with type 1 diabetes mellitus identified using corneal confocal microscopy. *Invest Ophthtalmol* Vis Sci 2016; 57: 853-8. DOI: 10.1167/iovs.15-18735
33. Shenoy R, Khandekar R, Bialasiewicz A, et al. Corneal endothelium in patients with diabetes mellitus: a historical cohort study. *Eur J Ophthalmol* 2009; 19:369-75. DOI: 10.1177/112067210901900307
34. Lee J, Oum B, Choi H, et al. Differences in corneal thickness and corneal endothelium related to duration in diabetes. *Eye* 2006; 20:315-8. DOI: 10.1038/sj.eye.6701868
35. Inoue K, Kato S, Inoue Y, et al. The corneal endothelium and thickness in type II diabetes mellitus. *Jpn J Ophthalmol* 2002; 46:65-9. DOI: 10.1016/S0021-5155(01)00458-0
36. Roszkowska AM, Tringali CG, Colosi P, et al. Corneal endothelium evaluation in type I and II diabetes mellitus. *Ophthalmologica* 1999; 213(4):258-61. DOI: 10.1159/000027431
37. Liaboe CA, Aldrich BT, Carter PC, et al. Assessing the impact of diabetes mellitus on donor corneal endothelial cell density. *Cornea* 2017; 36(5):561-6. DOI: 10.1097/ICO.0000000000001174
38. Vislisel JM, Liaboe CA, Waginer MD, et al. Graft survival of diabetic versus nondiabetic donor tissue after initial keratoplasty. *Cornea* 2015; 34(4):370-4. DOI: 10.1097/ICO.0000000000000378
39. Schwarz C, Aldrich BT, Burckart KA, et al. Descemet membrane adhesion strength is greater in diabetics with advanced disease compared to healthy donor corneas. *Exp Eye Res*. 2016; 153:152-8. DOI: 10.1016/j.exer.2016.10.015
40. Williams RS, Mayko ZM, Friend DJ, et al. Descemet membrane endothelial keratoplasty (DMEK) tissue preparation: a donor diabetes mellitus categorical risk stratification scale for assessing tissue suitability and reducing tissue loss. *Cornea* 2016; 35(7):927-31. DOI: 10.1097/ICO.0000000000000892

