Effects of Systemic Diseases on Graft Preparation in Descemet Membrane Endothelial Keratoplasty

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Abstract

Purpose: To evaluate the effects of the systemic diseases and drugs of the donor on Descemet's membrane (DM) graft preparation.

Methods: 78 corneas of 58 donors, of whom the DM grafts were used in Descemet's membrane endothelial keratoplasty (DMEK) surgery, between January 2018 – January 2020, were enrolled in this retrospective study. The hospital records of the donors were analyzed. Age, gender, blood type, systemic diseases, and drugs; complete blood count, biochemistry panel for liver and kidney functions in the last 48 hours, and the drugs used in the hospital, if any, in the last 24 hours were recorded. The grafts with tears that occurred while preparation, were included in group 1, and the successful grafts with no tears were included in group 2.

Results: There were no statistically significant differences in the characteristics of the donors between groups. However, the breast cancer and the use of sevelamer were found to be significantly higher in group 1 (p=0.010, p=0.033, respectively)

Conclusion: Although diabetic donors have been reported to be inappropriate candidates for the preparation of DM grafts for DMEK, most of the donors with several systemic diseases including diabetes can be used in DMEK surgery, with the right technique in DM graft preparation.

Introduction

Descemet's membrane endothelial keratoplasty (DMEK) is a lamellar keratoplasty, in which the posterior corneal layer is transplanted selectively with a lower rejection risk, a faster visual recovery, and a better anatomical restoration than penetrating keratoplasty. The procedure has been modified since it was first described in 2006 [1–3]. Surgically induced astigmatism and suture-related problems are minimal because of the minimal manipulation on the host anterior surface [4].

Several techniques have been described in the preparation of Descemet's membrane (DM) graft in DMEK [5–7]. Successful DM graft preparation without any tears is a critical step in DMEK surgery. It has been shown that the surgeon's skill and experience are the most important factors in dissection techniques and rescuing the central and peripheral tears in grafts with tight DM adhesions [8]. These tears in DM graft complicate the effective use of this delicate tissue of 10um in thickness. Apart from the surgical manipulation, also the structural differences in donor tissue have a role in the tears in DM grafts while preparing the tissue [9]. Moreover, the effect of the systemic disease and the drugs of the donor in a successful DM graft remain unclear.

This study aims to evaluate the effect of systemic diseases and drugs of the donor on DM graft preparation.

Materials And Methods

Seventy eight corneas of 58 donors, of whom the DM grafts were used in DMEK surgery in an ophthalmology clinic of a training and research hospital between January 2018 – January 2020, were enrolled in this retrospective study. The study was approved by the local ethics review board and adhered to the tenets of the Declaration of Helsinki.

The donors were harvested by the experienced personnel of our eye bank. The hospital records of the donors were analyzed. Age, gender, blood type, systemic diseases, and drugs; complete blood count, biochemistry panel for liver and kidney functions in the last 48 hours, and the drugs used in the hospital, if any, in the last 24 hours were recorded.

The video records of DM graft preparation were analyzed. All the grafts were prepared just before the procedure in the operating room, by the same experienced surgeon (YK). The grafts were divided into two groups. The grafts with tears occurred during preparation, were included in group 1, and the successful grafts with no tears were included in group 2.

The grafts from donors with a history of ocular surgery, Descemet folds and/or endothelial precipitates were not used for DM graft [10]. The donor corneas were preserved in Eusol-C (Corneal Chamber, Alchimia, Ponte San Nicolo, Italy) at + 4°C. DM graft preparation and DMEK surgery were performed in 7 days.

Graft preparation

The diameter of the graft was determined according to the diameter of the cornea from limbus to limbus of the recipient's eye. All grafts were prepared with the guidance of a technique described as submerged corneas using backgrounds away (SCUBA) [11]. Donor cornea was placed endothelial side up on a vacuum punch (Katena Products, Inc. New Jersey, USA). Trypan blue 0.06% solution was used to stain the edges of DM. Under the fluid, peripheral 180 degrees of DM was stripped, from trabecular meshwork to central cornea by using a crescent knife, trying not to form a tear. DM was grasped with a sinskey hook and 360 degrees of stripping was performed by using tying forceps. When nearly half of the DM was stripped, the graft was restained. Then, superficial partial-thickness trephination was performed from the endothelial side with a diameter that was determined before (7.50–8.50 mm). Then, DM was grasped again with a tying forceps and stripped until 360 degrees of DM was free from the stroma. Donor graft was restained by trypan blue 0.06% and aspirated into a glass injector system (DMEK Surgical Disposable Set, INNOVA Medical Ophthalmics, Toronto, Canada) in the fluid to deliver the anterior chamber of the recipient's eye (Fig. 1).

In case of a peripheral DM tear occurrence, while stripping from the trabecular side, the same procedure was repeated from the opposite side. If again a tear was formed then central punching was performed by not including the tear (Fig. 2). These grafts were included in group 1, the grafts without any tear formation were included in group 2.

Statistical Analysis

Data analysis was performed using the Statistical Package for Social Sciences for Windows software (SPSS version 16.0, SPSS Inc., Chicago, USA). The normality distribution of the variables was tested. The descriptive statistics of the normally distributed continuous variables were expressed as mean ± standard deviation. The normally distributed variables were compared between the groups, using a Student's t-test. Categorical variables were presented as frequency (%) and compared between the groups using the chi-square test or Fisher's exact test. Differences with a *p* value of < 0.05 were considered statistically significant.

Results

Seventy-eight corneal grafts (30 female, 48 male) from 58 donors were enrolled in the study. The mean age of donors was 64.23 ± 7,3 years. No statistically significant differences were found in the mean age and gender between groups (p = 0.920 and p = 0.480, respectively). 24 (31.2%) DM grafts were included in group 1. Tears occurred in 1 quadrant in 6 (7.8%), in 2 quadrants in 7 (9.1%), and 4 quadrants in 11 (14.3%) DM grafts. 53 (68.8%) DM grafts were

included in group 2.

The blood type of donors were A Rh + in 6 (10.5%), B Rh + in 5 (8.8%) and 0 Rh + in 5 (8.8%) donors in group 1 and A Rh + in 11 (19.3%), B Rh + in 10 (17.5%) and 0 Rh + in 5 (8.8%) donors in group 2. There was no statistically significant difference between groups (p = 0.450).

In 72 (93.6%) donors, at least one systemic disease was found, 23 (31.9%) in group 1 and 49 (68.1%) in group 2 (p = 0.570). The systemic diseases were summarized in Table 1. Of the donors with systemic diseases breast cancer was found only in group 1 (n = 6, 25%) and the difference was statistically significant between groups (p = 0.010) (Table 1).

Table 1
Systemic diseases of the donors

Systemic diseases	Group 1	Group 2	p value
Diabetes mellitus	8 (33.3%)	15 (28.3%)	0.655
Hypertension	9 (37.5%)	31 (58.5%)	0.08
Chronic renal failure	7 (29.2%)	11 (20.8%)	0.419
Hyperlipidemia	6 (25%)	16 (30.2%)	0.641
Osteoporosis	1 (4.2%)	5 (9.4%)	0.424
Breast cancer	6 (25%)	-	0.01
Endometrium cancer	-	4 (7.5%)	0.167
Prostate cancer	1 (4.2%)	1 (1.9%)	0.56
Lung cancer	2 (8.3%)	6 (11.3%)	0.691
Asthma and COPD	1 (4.2%)	9 (17%)	0.121
CHF	1 (4.2%)	2 (3.8%)	0.93
Venous embolism	3 (12.5%)	2 (3.8%)	0.15
COPD:Chronic Obstructive Pulmonary Disease, CHF:Congestive Heart Failure			

The use of systemic drugs was summarized in Table 2. Sevelamer use was only found in group 1 (n = 2, 8.7%) and the difference was statistically significant between groups (p = 0.030). No statistically significant difference in the use of other drugs was found between groups (Table 2).

Table 2
The systemic drugs of the donors

Systemic drugs	Group 1	Group 2	p value
Insulin	7 (30.4%)	7 (13.7%)	0.089
Metformin	5 (21.7%)	9 (17.6%)	0.677
Acetylsalicylate	5 (21.7%)	18 (35.3%)	0.244
Clopidogrel	4 (17.4%)	18 (35.3%)	0.119
Statin group	5 (21.7%)	15 (29.4%)	0.492
Paclitaxel and Carboplatin	7 (30.4%)	8 (7%)	0.144
5-fluorouracil and Cisplatin	2 (8.7%)	6 (8%)	0.694
Setron group	7 (30.4%)	7 (13.7%)	0.089
Levetiracetam	2 (8.7%)	4 (7.8%)	0.901
Thiazide group	2 (8.7%)	3 (5.9%)	0.655
Alpha-1 blockers	2 (8.7%)	3 (5.9%)	0.655
Metoprolol	5 (21.7%)	12 (23.5%)	0.865
Carvedilol	4 (17.4%)	12 (23.5%)	0.553
Ramipril	4 (17.4%)	11 (21.6%)	0.679
Furosemide	6 (26.1%)	8 (15.7%)	0.290
Amlodipine	2 (8.7%)	12 (23.5%)	0.132
Valsartan	4 (17.4%)	8 (15.7%)	0.854
Sevelamer	2 (8.7%)	-	0.033

There was no statistically significant difference between groups in the use of drugs in the last 48 hours for respiratory or cardiac arrest, such as dopamine, amiodarone, and adrenaline (p = 0.130). The increased levels above the normal range in BUN and creatinine were found to be higher in group 1 than group 2 (87.5% vs. 66%, p = 0.050). No statistically significant difference was found in increased levels of ALT and AST in donors when compared between groups (p = 0.984). Also, there was no statistically significant difference in lower hemoglobin levels between groups (p = 0.701).

Discussion

The first step of DMEK surgery is DM graft preparation [9]. One of the most concerning reasons in graft preparation is graft loss, whereas recent studies have reported successful outcomes with partially implanted grafts [11–13]. The most common cause of graft loss is the tears on the graft due to the tight

adhesions or increased fragility of the graft [14]. Although the procedure seems to be standardized, the peripheral tears in DM graft remain a potential risk. While small tears may be salvageable, an extra graft must be reserved in case of a large tear [15].

The differences in histochemical properties of the DM graft and the thickness of the graft may cause a change in the resistance at the peeling stage of the preparation step. The systemic diseases and drugs of the donor may also affect the elasticity and resistance of the tissue. The structural stress of the tissue increases due to the differences in adhesions between tissues. There is a narrow transition zone that includes an amorphous extracellular matrix, called interfacial matrix, between the DM and the stroma. Extracellular matrix and adhesive proteins, such as fibronectin, vitronectin, amyloid P, osteonectin/SPARC, fibrillin-1, fibulin-1 ve fibulin-3, keratoepithelin, collagen type VI, and VIII are found immunohistochemically in this zone [16, 17]. DM graft tears during peeling may be a result of these adhesive properties of the interfacial matrix. During DM graft preparation, adhesions between posterior stroma and DM may resolve spontaneously or result in DM tears if the tractional forces exceed the tensile adhesive strength [18]. The structural and biochemical differences between donors may cause the different adhesive forces between posterior stroma and DM [17].

Failure in DM graft preparation has been reported between 4.2–6.7% [8, 19–21]. The most commonly used technique in graft preparation is the SCUBA technique, in which the success rate has been reported over 95% [20, 22]. In this study, only the SCUBA technique is used to prepare the DM grafts. To eliminate the intersurgeon differences, the DM grafts were prepared by only one experienced surgeon. The first 200 cases of the surgeon were not included in the study to exclude the learning curve of the surgeon. As we know that with younger donors, the adhesive forces and the possibility of tears are higher, donors younger than 40 years old were also not included [23]. No age or gender-related factor was found to affect the failure rate of graft preparation in this study.

As far as we know, no investigation between blood type and DM tears was made. In this study, the highest rate of DM tears was found in donors with a blood type of A Rh+, but no statistically significant difference was found. This finding may be due to the higher rates of A Rh + blood type in our population.

Previous studies have shown that diabetes, hyperlipidemia, obesity, and chronic renal failure in the donors cause failure in preparation of the DM graft [19, 24]. It has been suggested that in diabetes, the tight adhesions between posterior stroma and DM may be the cause of DM tears [24]. In our study, no statistically significant difference was found in the rate of diabetes between groups. All the DM grafts from diabetic donors, whether intact or with tears were used in the surgery and no complications occurred. Moreover, no significant effect of the use of insulin and oral antidiabetic in donors was found on DM tears. In a study by Tien-en Tan et al., it has been shown 4.7% of failure in DM graft preparation and no effect of diabetes on DM tears. Thus, it is thought that not only metabolic diseases, such as diabetes but multiple structural differences may be related to the occurrence of DM tears while graft preparation [25].

It has been reported that systemic diseases, cause of death, and age of the donor may have a role in the failure of DM graft preparation [19, 26]. Among systemic diseases of donors, only the rate of breast cancer was found to be significantly higher in group 1. Increased inflammatory response, vascular endothelial dysfunction, increased vascular endothelial growth factor response, and increased oxidative stress are parts of the pathophysiological process of breast cancer [27, 28]. Thus, the increased oxidative stress may be the cause of the dysfunction of the DM-endothelium complex of the graft and leads to the failure of DM graft preparation.

When systemic drugs that were used more than 1 year were analyzed, only sevelamer use was found to be more common in group 1. Sevelamer is indicated in the control of hyperphosphatemia in patients on hemodialysis or periton dialysis. It is unclear, whether the use of sevelamer itself or the indication, hyperphosphatemia, is responsible for this finding.

While no statistically significant differences were found in anemia or liver function tests between groups, renal function tests (BUN, creatinine) were found to be more common in group 1. The level of urea in the aqueous humor has been shown to increase with increased urea levels in the serum [29]. The design of this study is not suitable to understand the effects of increased blood urea and creatinine levels on DM/endothelium complex so further studies are needed to reveal the pathophysiology.

The limitations of our study are its retrospective nature and the lack of a control group that includes naive donors without any systemic disease or use of any drug. However, it is almost impossible to find enough healthy donors in relatively old age within the criteria of donor bank to form a control group.

In conclusion, most of the death in elderly occurs with individuals with systemic diseases. On the other hand, the need for donor corneas for the patients in transplant lists is exponentially increasing. Therefore, to provide this shortage it would be effective to use all the available donors for transplant. Although diabetic donors have been reported to be inappropriate candidates for the preparation of DM grafts for DMEK, most of the corneas of the donors with several systemic diseases including diabetes can be used in DMEK surgery with the right technique in DM graft preparation.

Declarations

Funding This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (Adana City Training and Research Hospital, 29.01.2020, 49, 701).

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Figures

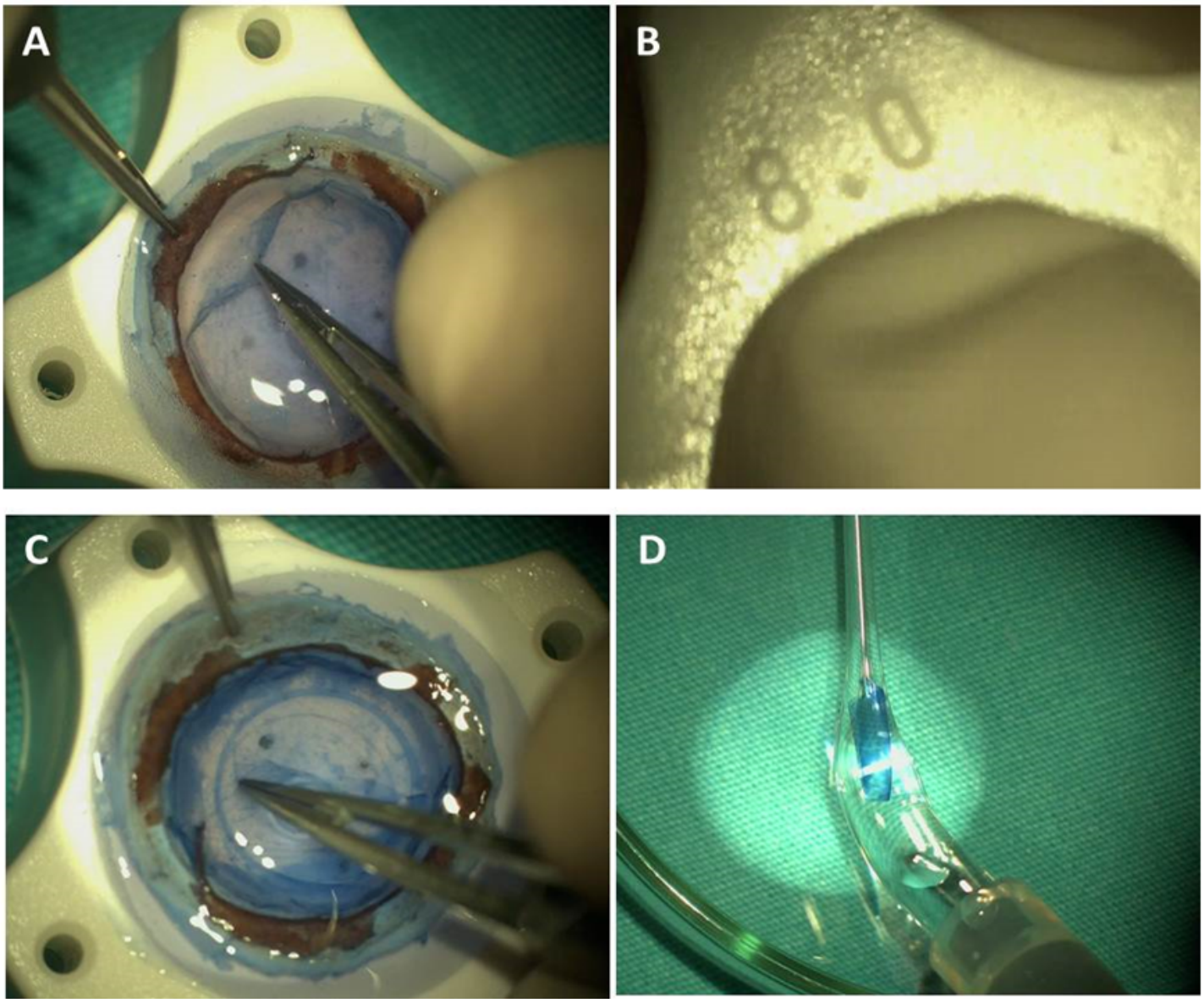

Figure 1

Steps of the preparation of a DM graft; (A) stripping the DM from the periphery (B) superficial partial-thickness trephination, (C) stripping of the rest of DM graft, (D) aspiration of DM graft into a glass injector system.

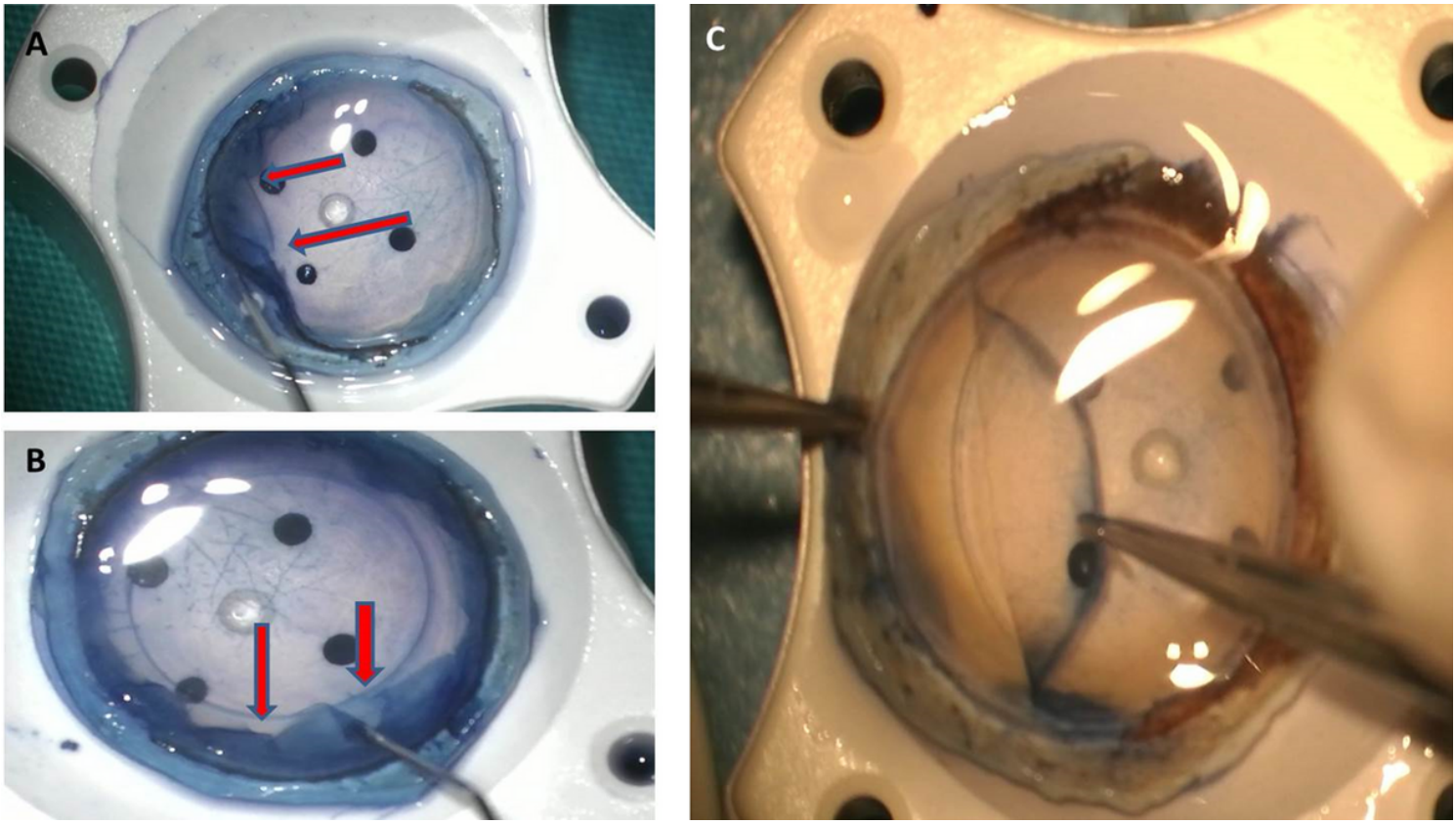

Figure 2

(A,B) Tight adhesions and tears from two cases in group 1, (C) example of successful preparation of a graft in a case in group 2.