Pathological Evaluation of Corneal Regrafts During Ten Years at a Tertiary Referral Center in Tehran, Iran

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Research Article

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Abstract

Purpose

To evaluate the histopathological and epidemiologic features of corneal regrafts over ten years.

Methods

A single-center retrospective analysis was performed on corneal specimens diagnosed as corneal graft failure retrieved from The Farabi Eye Hospital (Tehran, Iran) over a 10-year period. Also, Demographic data including sex, age, laterality, primary diagnosis, type of graft, cause of corneal regraft, associated eye diseases and systemic comorbidities were evaluated.

Results

Among a total of 3120 corneal grafts, 267 of them (8.55%) were regrafts. Main cause of graft failure was corneal ulcer. The most common type of grafting was penetrating keratoplasty (PK), but most common type of regrafting surgery was DSAEK. The Main causes of graft failure was Infectious keratitis. The most common histopathological finding was Bowman layer disruption (81%) and stromal scar (80%). Stromal edema, retro corneal fibrous, sub epidermal bulla and stromal vascularization were the other present findings. The inflammatory reaction was considered discrete in 59.45% of the cases and dominant cell type was polymorphonuclear neutrophils (PMNs).

Conclusion

Infectious keratitis was the major cause of failure in our study. Considerable presence of histopathological changes such as retro corneal fibrous, sub epidermal bulla needs further investigations. Perhaps, proper education of infectious keratitis risk factors, as well as regular examinations and proper antibiotic prophylaxis can reduce the rate of the corneal graft failure

Introduction

In recent years, on average, 180 000 corneal grafts are performed every year all over the world and has been continuously evolving in recent years, by the introduction of new posterior lamellar techniques. the graft failure, defined as any irreversible change in the graft preventing recovery of useful vision, still represents a consistent concern, despite these continuous improvements during this decades.[1–3]

Corneal grafts survival depends on many factors such as the quality of the donor tissue (corneal storage, and eye bank procedures), the surgery technique, postoperative care, and rapid controlling and managing of the complications occurring afterward such as infection. [2, 4–6]
Postoperative complications of the corneal graft surgery include trapping of the corneal wound, wound leakage, endophthalmitis, glaucoma, corneal endothelial cells impaired function, persistent epithelial defect, primary disease recurrency, and stromal or endothelial rejection. Complications of graft sutures include tight fastening or loosening of the sutures, noninfectious infiltration of the sutures, infectious abscess, and vascularization along the incision and sutures. Bacterial, viral, and fungal graft infections are among other serious complications after corneal graft. [5, 6]

All mentioned consequences and complications, if not treated properly on effective time, and simultaneous occurrence with other factors such as ocular trauma, ocular surface diseases, eyelid disorders, systemic diseases with ocular manifestations such as autoimmune diseases [e.g., rheumatic arthritis], hypothyroidism, and previous intraocular surgeries like glaucoma surgery can threaten the grafted tissue and results in pain, bother and finally unfavorable vision. [5, 7–8]

In this study we evaluate the histopathological and some epidemiologic findings of corneal regrafting conducted during 10 years (2012–2022) at Farabi Eye Hospital, Tehran, as the tertiary referral center in Iran, in order to find the main histopathological characteristics of corneal regrafts based on histologic findings for better understanding of pathophysiology of graft failures.

**Methods**

In the present retrospective cross-sectional study, histopathological and epidemiological characteristics of all corneal regrafts at Farabi Eye Hospital, Tehran, Iran were evaluated for 10 years (2012–2022). Approval for the study was obtained from the ethical committee of the Tehran University of Medical Sciences which complies with the Helsinki Declaration.

Firstly, all corneal graft files in the abovementioned years were assessed. The following parameters were obtained from medical charts: date of the diagnosis of the corneal graft failure, age at the time of the regraft, sex, gender, area of residence (province and urban versus rural) interval between surgeries (first and second graft survival), the reason for regraft, history of coexisting eye disease, and presence of systemic comorbidity were recorded in the designated forms. Graft failure was defined as an irreversible loss of central graft clarity using a slit-lamp biomicroscope examination. sixty-seven cases of corneal regraft were found, and data were retrieved from their files.

Specimens were fixed in a 10% buffered paraformaldehyde solution for 24 hours, bisected through the center of the button, and embedded in paraffin. Sections of 5 mm were stained with hematoxylin and eosin and periodic acid–Schiff hematoxylin and eosin. all slides were reviewed by an expert ocular pathologist using an Olympus BX40 microscope.

Histological findings were evaluated, including:

- Epithelial layer status: Normal, atrophic, hyperplastic, sub-epidermal bulla, sub-epithelial fibroblastic scar
Bowman layer status: Disrupted, fibrosis, normal

Endothelial layer Status: Normal, a decrease of cellular size, a decrease of cell density, endothelial layer destroyed (absent)

Descemet layer status: Normal, disrupted, increase of thickness, thinning, Doubling and excretions, retro corneal fibrous ingrowth

Stromal layer status: Presence of scar, deposits, necrosis, acute or chronic inflammation, vascularization

Statistical analysis was performed using SPSS software version 24 (SPSS Inc., IBM). Data are presented as mean ± standard deviation and percentages. the chi-square test was used for the comparison of data. For all statistical analyses, a P value of less than 0.05 was considered statistically significant.

Results

During 10 years (2012–2022), 3120 cases of corneal transplants were conducted at Farabi Eye Hospital, Tehran. Among them, 2018 (64.67%) were penetrating keratoplasty (PK), 640 (20.51%) were Descemet stripping endothelial keratoplasty (DSAEK), and 462 (14.80%) were deep anterior lamellar keratoplasty (DALK). Hence, among 2018 cases of PK during 10 years, 168 (8.32%) ended up with regrafting. However, the tissue samples of 34 patients were available for pathological evaluation. and among 640 cases of DSAEK and 462 cases of DALK, only 94 (14.68%) and 5 cases (1.08%) resulted in regrafting, respectively. Among regrafting DSAEKs, only 14 (2.18%) cases underwent regrafting with PK.

Finally, only two samples of DSAEK regrafting with PK and one sample of DALK cases were available for pathological evaluation.

The overall rate of corneal regraft was 8.55% (267 cases) during ten years, and among them, 180 cases (67.4%) were men. There was a statistically significant difference between males and females (P value < 0.01).

The average age at the time of corneal regraft surgery was 50 years with a standard deviation (SD) of 21.82. Also, the highest and lowest age was 89 years and 8 years, respectively.

Regarding the residential area, Northern and coastal Province had the largest number of referrals (130 out of 267), followed by Southern states with warm climates (64 cases), Khorasan province (8 cases), and Fars’s province (5 cases). The rest were from other neighboring provinces of Tehran.

The most prevalent cause of primary corneal transplantation in patients who had later undergone corneal regraft surgery was the corneal infection or corneal ulcer [fungal (18.2%), herpetic (13.8%), and bacterial (10.3%)] that followed by aphakic bullous keratopathy (32.2%). (Table 1). Infective causes altogether (42.3%) were the main causes of primary corneal graft in regraft cases (113 cases). Among all graft failures that resulted in regrafting, recurrence of primary disease was the cause of failure in 34 cases (14
fungal corneal ulcers, 10 herpetic infections, 7 bacterial corneal ulcers, and 3 corneal dystrophies) during 8–10 months after primary surgery.

<table>
<thead>
<tr>
<th>Causes of regraft</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious keratitis (corneal ulcer)</td>
<td>113</td>
<td>42.3</td>
</tr>
<tr>
<td>Endothelial dysfunction &amp; corneal decompensation</td>
<td>86</td>
<td>32.2</td>
</tr>
<tr>
<td>Rejection</td>
<td>38</td>
<td>14.2</td>
</tr>
<tr>
<td>Corneal dystrophy</td>
<td>18</td>
<td>6.7</td>
</tr>
<tr>
<td>Technical problem</td>
<td>12</td>
<td>4.5</td>
</tr>
<tr>
<td>Total</td>
<td>267</td>
<td>100</td>
</tr>
</tbody>
</table>

The secondary surgical procedure in the vast majority of cases 70% (187 out of 267 regrafts) was PK; 30% (80 out of 267 regrafts) underwent re-DSAEK. In 12 DSAEK cases, re-DSAEK was needed due to the technical problem of the primary surgical procedure; all re-DSAEKs were performed between 1–8 months after the primary operation.

the average interval between the first graft and regraft procedures was 56 months. the shortest interval was 1 month (re-DSAEK was carried out because of failed DSAEK). The longest graft survival also belonged to PBK cases. Systemic comorbidity [16.2% hypertension, 10.8% diabetes mellitus, 8.1% ischemic heart disease, and 8.1% other disorder] was found in 43.2% of the patients.

In regards to microscopic evaluation, only 37 regraft cases underwent pathological evaluation.

Epithelial changes were observed in 51.3% of cases including [ hyperplastic (32.4%), subepithelial bulla (29.7%, Fig. 1.A), thinning(atrophic) (18.9%), and sub-epithelial fibroblastic scar (27%, Fig. 4. A)]. The results of the chi-square test showed that there is no statistically significant relationship between epithelial abnormalities and graft failure resulting in corneal regrafts (P = 0.068).

Bowman layer changes were observed in 100% of cases [ including, disrupted bowman layer (80%) and bowman layer fibrosis (20%)]. According to chi-square analysis, there was a statistically significant relationship between Bowman layer abnormalities (especially disrupted bowman layer) and graft failure resulting in corneal regrafts (P = 0.001).

Endothelial layer abnormality was observed in 78.3% of cases. These abnormalities included endothelial cell loss (74%), and complete endothelial layer absence (26%). There was a statistically significant relationship between endothelial layer changes (especially endothelial cell loss) and graft failure resulting in corneal regrafts (P = 0.001).
Descemet layer changes were seen in 83.7% of the failed graft samples [Descemet layer disruption (43.2%), retro corneal fibrosis (27%, Fig. 2 and Fig. 3.A), thickening (18.9%), thinning (8.1%), doubling and excretions (8.1%). There was a statistically significant relationship between Descemet layer abnormality and graft failure resulting in corneal regrafts (P = 0.002).

Stromal layer abnormality was observed in 81% of failed graft cases. These abnormalities include: stromal scar (81%), stromal edema (56.7%, Fig. 1.A), stromal vascularization (35.1%, Figs. 3 & 4B), chronic inflammation (29.7%), acute inflammation (24.3%) and stromal degeneration (5.4%). The predominant inflammatory cell in the stromal layer was PMNs. There was a statistically significant relationship between stromal layer changes and graft failure resulting in corneal regrafts (P = 0.002).

Histopathological findings of all 37 corneal regraft samples are given in Table 2.
Table 2
Histopathological findings of corneal regrafts

<table>
<thead>
<tr>
<th>Corneal layers</th>
<th>Pathological findings</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyperplastic</td>
<td>12</td>
<td>32.4</td>
</tr>
<tr>
<td>Epithelium</td>
<td>Sub epithelial bulla</td>
<td>11</td>
<td>29.7</td>
</tr>
<tr>
<td></td>
<td>Thinning</td>
<td>7</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>Sub-epithelial fibroblastic scar</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Bowmen</td>
<td>Disruption</td>
<td>30</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Fibrosis</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Endothelium</td>
<td>Endothelial cell loss</td>
<td>27</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Complete absence</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Descemet layer disruption</td>
<td>16</td>
<td>43.2</td>
</tr>
<tr>
<td></td>
<td>Retro Descemet fibrous</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Descemet</td>
<td>Thickening</td>
<td>7</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>Thinning</td>
<td>3</td>
<td>8.10</td>
</tr>
<tr>
<td></td>
<td>Doubling and excretions</td>
<td>3</td>
<td>8.10</td>
</tr>
<tr>
<td></td>
<td>Stromal scar</td>
<td>30</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Stromal edema</td>
<td>21</td>
<td>56.7</td>
</tr>
<tr>
<td>Stroma</td>
<td>Stromal vascularization</td>
<td>13</td>
<td>35.1</td>
</tr>
<tr>
<td></td>
<td>Stromal degeneration</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Chronic inflammation</td>
<td>11</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Acute inflammation</td>
<td>9</td>
<td>27.3</td>
</tr>
</tbody>
</table>

**Discussion**

Corneal transplantation is one of the important therapeutic options to restore vision in some corneal diseases. Although the survival rate of corneal graft is still affected by serious causes, the initial success of corneal graft is close to 90%. In recent decades, the frequency of corneal regrafts has increased. This can probably be attributed to the greater availability of donor tissue resulting from increasing eye-banking activities and the growth of the number of corneal transplantations. [9–12]

In this study, the mean age of the regraft recipients was (50.9 ± 21.8 years) similar to another study from Brazil and Canada. [13, 15] Weisbrod et al showed that the mean age of their patients that underwent
regraft surgery was 5 years older than those undergoing corneal graft for the first time. More corneal graft failures at younger ages could have been either because of a higher proportion of complicated cases [e.g., corneal dystrophy, ocular trauma] or poor compliance with postoperative care among younger ages. [13, 15] The sex ratio in our study was similar to the Indian study where 68% of the patients were men [15, 16], but not follow the others studies realized in developed countries. [13, 14]

Our study showed that the rate of corneal regraft in all transplanted corneas during ten years was 8.55% which is similar to the corneal graft failure rate (5–30%) in other studies [7, 8, 17, 18]. In this study, DALK was the most successful type of grafting with a regraft rate of 1.08% followed by PK (8.32%) and DSAEK (14.98%). Most probably reasons for the acceptable rate of regrafting in DALK cases were younger age at the time of surgery, the nature of the main disease (keratoconus in most of our DALK cases), and retaining recipients’ endothelium. Considering the time interval between the primary graft and regrafting (1.5–6 months), the failure was most probably caused by technical problems [19]. Considering the strict DSAEK technique and less familiarity of our cornea surgeons with the DSAEK technique at the beginning years of DSAEK introduction, the success rate was still very acceptable in our tertiary center. Other reasons for the higher rate of re-DSAEK in our center can be attributed to the special socio-climatic conditions that prone the most patient to ocular surface complications, and due to the higher prevalence of ocular surface problems in PK patients, it has been attempted in recent years even in most cases of PK failure due to endothelial dysfunction, primary regraft surgery is DSAEK.

In our study, in contrast to previous studies, infectious causes (corneal ulcer) of the grafted cornea were the main cause of regrafting [4, 5, 17]; however, following most studies, endothelial dysfunction had a more significant role in corneal regraft [5, 6, 17, 18]. It can be due to climacteric differences or social-cultural conditions of most of our cases who are not able to follow the recommended medical care.

Based on this study, the primary disease was proposed to be the most important indicator of graft survival. In our study, infectious keratitis (bacterial, herpetic, or fungal) was the major cause of second and even in some patients’ third graft surgery. In our study, 34 cases had a recurrence of infection. This finding highlights the risk of primary infection recurrence in grafted corneas and emphasizes the importance of antiviral treatments or continuing proper antibiotics after corneal transplantation. Also, a recent cohort study showed that, despite the various changes in the corneal grafting techniques in the past decades, the trephination size and location have no considerable impact on graft survival compared with other factors. However, accurate surgical techniques regarding the location and trephination size help to remove the main focus of infection while keeping a safe margin to the limbal area can reduce the primary infection recurrency in grafted tissue [19].

The quality of donor tissue may also be an influential factor in graft survival. At this tertiary center, the higher quality tissues are preserved for optical keratoplasties [DALK & DSAEK], while moderate quality donor corneas are acceptable for emergency cases and tectonic purposes [e.g., corneal ulcer]. Reasonably, donor tissue with lower quality is prone to fail more easily than higher quality ones. However, this cannot be a convincing reason for reinfection and recurrence.
In our study, diabetes and hypertension were found in 16.2% and 10.8% of the patients; previous studies considered that diabetes is a major risk factor for corneal graft failure [20].

The histopathological evaluation of the regrafting cases showed that the epithelial layer changes were widely observed in accordance with previous studies [20, 21]. Also, following previous studies, the existence of thinning and thickening of the epithelial layer was observed in 32% and 18.9% of regrafts, which may have contributed to corneal graft failure [21]. Another interesting finding in our study was the high number of Sub epithelial bulla in failed graft cases.

Bowman's layer disorder was the most common histopathological finding in our study (100%), especially, Bowman's layer Disruption which has been reported in the previous study [21, 22]. Bowman’s layer disorders in our study were associated with graft failure.

Stromal scar and edema in the present study were observed in the majority of cases (81% & 56%), especially in the infectious keratitis associated with corneal graft failure. This histological finding was considered a risk factor for graft failure, and this stromal finding was in accordance with previous studies [20, 21, 22]. Stromal vascularization was observed in 35% of cases, and this finding was observed in previous studies too.

In our study, the inflammatory reaction was graded acute and chronic based on the extent of the inflammatory cell infiltration. The majority of cases had at least some inflammatory reaction within the stroma. Previous studies have shown that newly recruited bone marrow-derived--inflammatory cells produce angiogenic factors and therefore may play role in the mechanisms that induce hemangiogenesis in corneal tissue [23, 24]. Our results about the presence of more stromal vascularization in failed graft tissues that had acute and chronic inflammation may confirm this correlation.

The Descemet layer disruption and retro Descemet fibrous were interesting finding in our study. Also, doubling and excretions of the Descemet layer were observed in some cases and this finding was observed in previous studies [21].

In our study, endothelial cell loss was observed in the majority of cases which is following previous studies [20,21,22]. The endothelial cells are the main target of an immune-mediated attack during a corneal failure reaction [25]. Corneal endothelial cells have no mitotic capacity but have a major role in the process of wound healing by spreading out. Endothelial cells on grafted corneas compared with that of healthy corneas are lost at accelerated rates [25, 26]. Bertelmann et al reported that the mean endothelial cell loss after PK was 28.8% and 39.8%, after 6 and 12 months, respectively [28]. The endothelial cell loss affects the ability of the endothelium to maintain its main function, which eventually leads to a hazy graft [26, 28].

Our study had an important limitation. Despite the high number of regrafting cases, tissue samples were available for histopathological evaluation in limited cases (only 37 cases). This limitation can be due to
several reasons, such as a definitive diagnosis of failed graft pathology based on imaging modalities such as confocal and specular microscopy.

**Conclusion**

In conclusion, our results provide histopathological evidence for the diagnosis and treatment of corneal graft failures. As regards, infectious keratitis being the major cause of graft failure, the infection should be controlled more vigorously by proper medication after transplantation. Also, the patients should be warned about complications, infectious keratitis risk factors, and the need for regular follow-ups.

**References**


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Figures

![Figure 1](image-url)
Corneal graft failure, A: corneal tissue shows sub epithelial bulla formation (arrow). B: stromal edema (arrowhead). medium magnification H&E stain.

**Figure 2**

Corneal failed graft tissue show retro-corneal fibrosis (arrow), Low and medium magnification. H&E stain

**Figure 3**
A, B; Corneal failed graft tissue shows retro-corneal fibrosis (arrow), vascularization (arrowhead) and acute inflammatory cells infiltration(stars). Low and medium magnification. H & E stain

Figure 4

Corneal graft failure. A: corneal tissue shows sub-epithelial fibroblastic scar(arrow). B: vascularization (arrowhead) and acute inflammatory cells infiltration(star). Low and medium magnification. H & E stain.