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The role of Descemet's membrane in the pathogeny of corneal edema following anterior segment surgery

O papel da membrana de Descemet na patogenia do edema corneano após cirurgia de segmento anterior

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ABSTRACT

O**bjective:** To find relevant factors in the pathogeny of postoperative corneal edema in post-cataract surgery and post-keratoplasty cases, through the study of histopathological findings in order to see what can be done to avoid successive keratoplasties. **Methods:** Retrospective descriptive study of histopathological findings in postoperative corneal edema cases. Tissues were obtained from penetrating keratoplasty in the period between september 2009 and august 2013. A medical record review was conducted primarily looking for information about previous surgeries. **Results:** Seventy corneal buttons were included, out of which 34 were from male patients and 36 were from female patients. The mean age was 63.1±17.20 (mean ± SD) years. Most of cases were corneal failure after keratoplasty (71.43%). The main change found in endothelium was cellular rarefaction (58 cases), and it was also the most common histopathological change. Changes in integrity predominated in Descemet's membrane (53 cases), whether in the form of rupture, isolated detachment or detachment associated with rupture. Endothelial changes associated with the absence in Descemet's membrane integrity were frequent. **Conclusion:** Descemet's membrane detachment is a frequent histopathological finding in postoperative corneal edema cases studied, thus it should be considered an important factor in the pathogeny of such cases. This change should be carefully researched in the postoperative period in order to be diagnosed and treated early, possibly avoiding many keratoplasty indications.

Keywords: Descemet membrane; Corneal edema/etiology; Corneal edema/pathology; Keratoplasty

RESUMO

Objetivo: *Encontrar fatores importantes na patogenia do edema corneano pós-cirúrgico, em casos de pós-facectomia e pós-ceratoplastia, por meio do estudo dos achados histopatológicos, a fim de ver o que pode ser feito para evitar sucessivas ceratoplastias.* ***Métodos:*** *Estudo retrospectivo descritivo das alterações histopatológicas de casos de edema corneano pós-cirúrgicos. Os tecidos foram provenientes de ceratoplastia penetrante no período compreendido entre setembro de 2009 e agosto de 2013. Foi realizada revisão de prontuários em busca principalmente de informações sobre cirurgias prévias.* ***Resultados:*** *Foram incluídos 70 botões corneanos, sendo 34 de pacientes do sexo masculino e 36 do sexo feminino. A média das idades foi de 63,1±17,20 (média ± DP) anos. A maioria dos casos era de falência após transplante (71,43%). A rarefação celular foi a principal alteração encontrada no endotélio (58 casos), sendo também a alteração histopatológica mais frequente. Na membrana de Descemet, predominaram as alterações de integridade (53 casos), seja na forma de ruptura, de descolamento isolado ou de descolamento associado à ruptura. Foi frequente a associação de alterações endoteliais à ausência da integridade da membrana de Descemet.* ***Conclusão:*** *Descolamento da membrana de Descemet é um achado histopatológico frequente nos casos de edema corneano pós-cirúrgicos estudados, devendo ser considerado um fator importante na patogenia dos mesmos. Essa alteração deve ser procurada com atenção nos pós-operatórios, a fim de ser diagnosticada e tratada precocemente, vindo possivelmente a evitar muitas indicações de ceratoplastia.*

Descritores: *Lâmina limitante posterior; Edema da córnea/etiologia; Edema da córnea/patologia; Ceratoplastia*

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Introduction

Corneal oedema is a clinical condition characterised by increased anteroposterior corneal thickness or diameter[1,2].It can be confined to the epithelium or part of it, and it can also affect only the stroma. Nevertheless, in most cases both the epithelium and the stroma are affected[2].

Wherever oedema develops, structural elements are separated from one another. In the stroma, the keratocytes are separated from each other and from adjacent collagen fibres; these, in turn, also move apart from one another. Oedema is generally associated with loss of transparency of the affected area of the cornea[2].

The two main factors in the aetiology of corneal oedema are endothelial dysfunction[2-4] and Descemet's membrane defects. Endothelial cells form a single layer of pump cells in the posterior cornea and are responsible for constantly dehydrating the cornea. These cells decrease in number with age and are usually incapable of regeneration[5].Ophthalmologists are well aware of the limited regeneration capacity of the human corneal endothelium and of the need for extreme caution to avoid injuries to this important tissue during surgical procedures[6].

Defects in Descemet's membrane also lead to the inflow of aqueous humour into the cornea, with subsequent stromal oedema[5].Descemet's membrane detachment can result from any factor causing a rupture in Descemet's membrane, including surgical procedures (cataract extraction, iridectomy, cyclodialysis, penetrating keratoplasty) and tears due to congenital glaucoma, keratoconus, keratoglobus, or trauma[7].Large and extensive detachments have an exuberant presentation, with corneal oedema and marked reduction in visual acuity, and often require surgical intervention to prevent permanent corneal decompensation and the need for corneal transplantation[8].

Given the above, our objective was to determine the pathogeny of postoperative corneal oedema after cataract extraction and keratoplasty by studying histopathology findings in Descemet's membrane and the endothelium, in order to determine potential early measures that might prevent the need for repeated keratoplasties. Histopathological changes may be suggestive of what needs to be rigorously investigated during the postoperative period in patients submitted to these procedures.

Methods

A study of corneal buttons from penetrating keratoplasties conducted between September 2009 and August 2013 and submitted to the Eye Bank for histopathological examination was conducted. Tissues were fixed in 10% neutral formaldehyde and sliced by a pathologist while lying on the endothelial side, with the cut starting from the epithelium and ending at the endothelium. Samples were embedded in paraffin, cut into 2.5-ìm thick slices, and stained with haematoxylin-eosin. Following preparation, tissues were examined by the authors with an optical microscope.

This was a retrospective, descriptive study of the histopathological changes found in postoperative corneal oedema. Because of the lack of studies to which our results could be compared, we simply assessed the frequency of each finding. Using this statistical method is also warranted by our interest in studying the pathogeny of the condition and examining the changes found in the sample. In further studies based on these findings, statistical probabilities can also be calculated.

Corneal buttons presenting stromal oedema were selected. Stromal oedema was histopathologically defined as the presence of separation between structures such as keratocytes and collagen fibres. Oedema was classified into five grades depending on the degree of separation. Epithelial oedema (cell oedema, cytoplasmic vacuoles, microcysts, and bubbles) was not considered in the classification, despite being present in almost all cases.

Specimens with grade I (mild) oedema presented only a slight and sparse separation between structures (Figure 1a).Cases where the separation was more frequent and widespread were classified as grade II (mild-moderate) oedema (Figure 1b). Grade III (moderate) oedema showed generalised swelling, but with slight separation between structures (Figure 1c). In grade IV (moderate-severe) oedema, the separation was wider and very frequent (Figure 1d). Finally, specimens with grade V (severe) oedema presented greater separation with generalised swelling (Figure 1e). In some cases, a tissue sample presented oedema of varying intensity in different regions. Such cases were classified according to the predominant type of oedema or to its average intensity.

The exclusion criteria were inflammatory reactions, synechiae, stromal thinning, and specimens without a medical record or with no record of prior surgery. Thus, only pure cases of postoperative corneal oedema were included, i.e. those not associated with secondary causes.

The medical records of selected cases were reviewed for

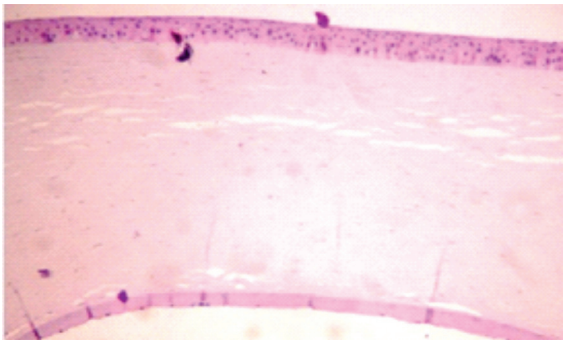


Figure 1a: Mild oedema (grade I) with slight, sparse separation of stromal structures. Note the thickened Descemet's membrane.

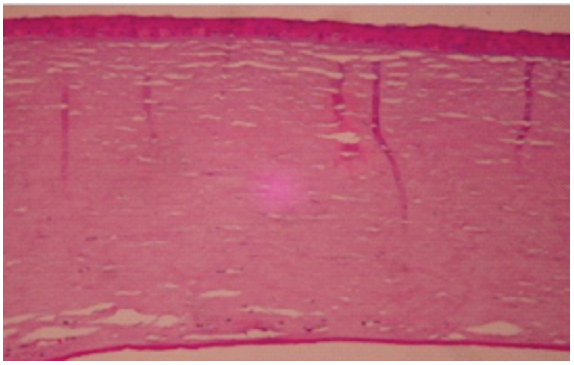


Figure 1b: Mild-moderate oedema (grade II) with more widespread separation.

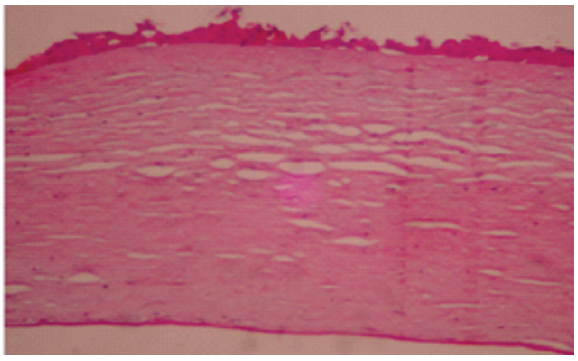


Figure 1c: Moderate oedema (grade III). The oedema is more generalised but the separation is still mild. Microcysts can be seen in the epithelium.

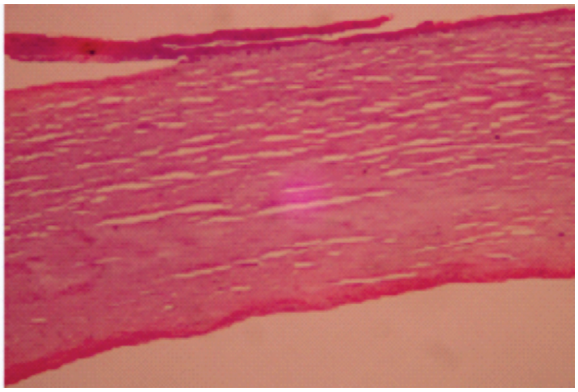


Figure 1d: Moderate-severe oedema (grade IV), with wider and very widespread separation. A large bubble can be seen detaching part of the epithelium.

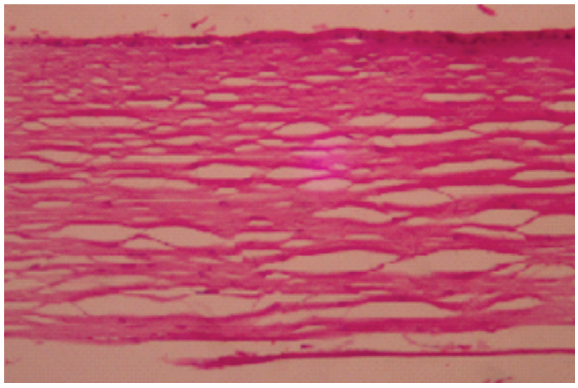


Figure 1e: Severe oedema (grade V). The oedema is generalised and the separation is much wider. Bubbles can be observed in the epithelium.

information on the surgeon's diagnosis, previous surgery, gender, and age. All cases of prior cataract extraction or corneal transplantation were recorded. Cases where the surgeon's diagnosis indicated bullous keratopathy following cataract extraction or graft failure following a previous transplantation were considered as such. In cases where the surgeon's diagnosis was lacking, this information was obtained in the medical record which contained a standard form for corneal transplantation. Cases of previous transplantation were considered as graft failure, and cases of previous cataract extraction were considered as bullous keratopathy.

During histopathological examination, samples presenting any degree of corneal and diagnosed with bullous keratopathy or graft failure were investigated for changes in Descemet's membrane and the corneal epithelium. Changes in Descemet's membrane included ruptures, detachment, folds, bifurcations, abnormal thickness, and distension. Endothelial changes included cell rarefaction or cell abnormalities. Since this study was focused on the pathogeny of corneal oedema, data collection was centred on its potential causal factors. Nonetheless, factors that seemed to be a consequence of the condition are also mentioned, in order to present the full array of histopathological findings.

Results

Seventy corneal buttons were included in this study, of which 34 were from male patients and 36 from female patients. Mean age was 63.1 ± 17.20 years (mean ± SD). The sample included 20 cases of bullous keratopathy following cataract extraction and 50 cases of graft failure. The distribution of cases according to the five grades of oedema is shown in Table 1.

The histopathological changes found in the samples are shown in Table 2. The most common changes in Descemet's membrane were ruptures in 41 cases (58.57%) and detachment in 40 cases (57.14%), followed by folds in 25 cases (35.71%). The main change found in the endothelium was cell rarefaction, in 58 cases (82.86%). Considering all types of changes, endothelial cell rarefaction was the most common.

Table 1

Sample distribution according to the grade of corneal oedema found in specimens submitted for examination between September 2009 and August 2013.

Grade	Bullous keratopathy following cataract extratction (%)	Graft failure (%)	Total (%)
I	5 (25)	13 (26)	18 (25.71)
II	3 (15)	8 (16)	11 (15.71)
III	4 (20)	7 (14)	11 (15.71)
IV	5 (25)	14 (28)	19 (27.14)
V	3 (15)	8 (16)	11 (15.71)
Total	**20 (100)**	**50 (100)**	**70 (100)**

The distribution of histopathological changes found in cases of bullous keratopathy following cataract extraction and graft failure are shown in Table 3.Ruptures and detachment were the predominant changes in Descemet's membrane, both for bullous keratopathy and graft failure. Cell rarefaction was the main endothelial change in both groups, as well as the most frequent histopathological change overall.

The charts below show the distribution of histopathological changes along the five grades of corneal oedema following anterior segment surgery. Only those changes that can be considered as causal factors are shown, namely changes to the integrity of Descemet's membrane (ruptures, ruptures plus detachment, and isolated detachment) and isolated endothelial changes. Chart 1 shows that changes to the integrity of Descemet's membrane were the predominant finding, with detachment being very frequent, whether or not associated with rupture (40 cases).

Table 2
Corneal changes found in specimens submitted for examination between September 2009 and August 2013.

Changes		Quantidade (%)
Descemet's membrane	Ruptures	41 (58.57)
	Detachment	40 (57.14)
	Folds	25 (35.71)
	Bifurcations	3 (4.29)
	Thickening	4 (5.71)
	Thinning	6 (8.57)
	Distension	3 (4.29)
Endotheliun	Cell rarefaction	58 (82.86)
	Cellabnormalities	9 (12.86)

Percentages do not add up to 100% because some specimens contained multiple changes.

Table 3
Comparison of changes found in cases of bullous keratopathy following cataract extraction and graft failure in specimens submitted for examination between September 2009 and August 2013.

Changes		Bullous keratopathy following cataract extratction (%)	Graft failure (%)
Descemet's membrane	Ruptures	10 (50)	31 (62)
	Detachment	14 (70)	26 (52)
	Folds	4 (0)	21 (42)
	Bifurcations	0 (0)	3 (6)
	Thickening	3 (15)	1 (2)
	Thinning	3 (15)	3 (6)
	Distension	0 (0)	3 (6)
Endotheliun	Cell rarefaction	15 (75)	43 (86)
	Cellabnormalities	1 (5)	8 (16)

Percentages do not add up to 100% because some specimens contained multiple changes.

Table 4
Changes to the integrity of Descemet's membrane in cases of postoperative corneal oedema.

Changes	N (%)
Ruptures	13 (18.57)
Detachment	28 (40)
Folds	12 (17.14)

Percentages do not add up to 100% because this table only includes changes to Descemet's membrane, which were not found in all specimens.

Isolated endothelial changes were only found in 17 cases. The distribution of changes in both groups was relatively similar to the sample as a whole (Chart 2).

The frequency of changes to the integrity of Descemet's membrane, either in isolation or associated with other findings, are shown in Table 4.The most common change was detachment associated with rupture, with 28 cases (40%). Isolated ruptures were found in 13 cases (18.57%), and isolated detachment was found in 12 cases (17.14%).

Considering the distribution of changes according to the grade of oedema (Charts 3-7), it was found that changes to the integrity of Descemet's membrane prevailed in all grades. A frequent change to the integrity of Descemet's membrane in all grades of oedema was detachment, whether or not associated with rupture. Isolated endothelial changes were found mainly in grade IV oedema, with 9 cases (47.37%), followed by detachment, found in 8 cases (42.10%).

Discussion

This was a descriptive study of histopathological changes found in Descemet's membrane and the corneal endothelium of patients with corneal oedema following anterior segment surgery (keratoplasty or cataract extraction). Most cases included in the sample were graft failures, which can be attributed to the fact that most corneal buttons submitted to the Eye Bank for histopathological examination come from emergency keratoplasties, where the corneal button is routinely submitted

Chart 1
Distribution of histopathological changes according to the grade of oedema.

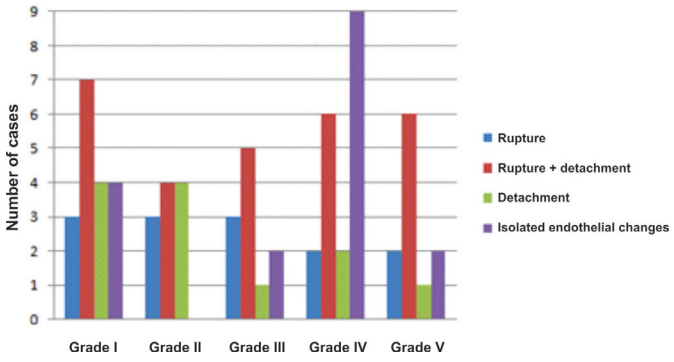


Chart 2
Histopathological changes found in cases of bullous keratopathy following cataract extraction (black) and graft failure (grey).

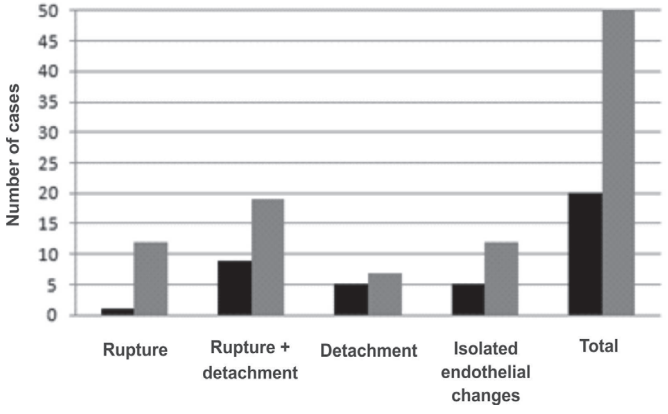


Chart 3

Histopathological changes found in cases of bullous keratopathy following cataract extraction (black) and graft failure (grey) with mild (grade I) oedema.

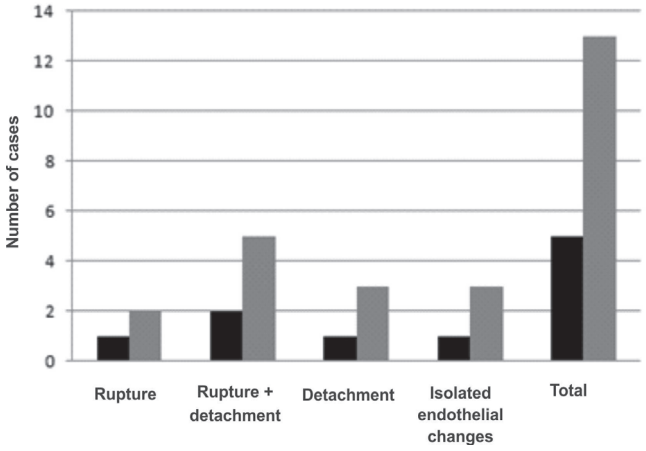


Chart 4

Histopathological changes found in cases of bullous keratopathy following cataract extraction (black) and graft failure (grey) with mild-moderate (grade II) oedema.

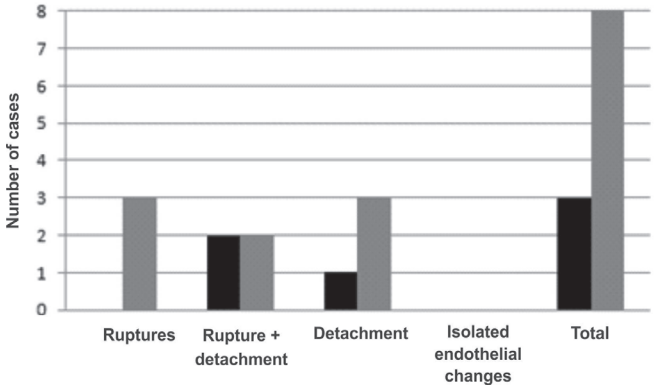


Chart 5

Histopathological changes found in cases of bullous keratopathy following cataract extraction (black) and graft failure (grey) with moderate (grade III) oedema.

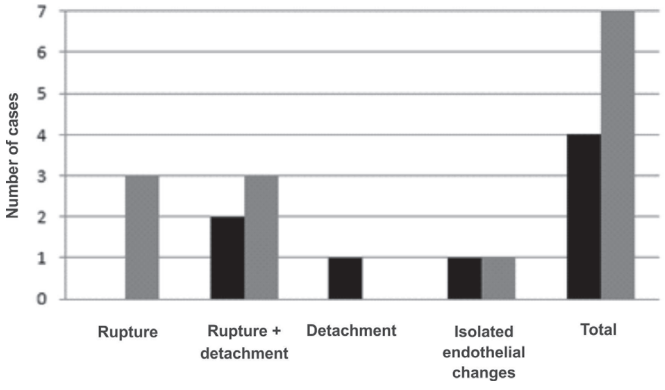


Chart 6

Histopathological changes found in cases of bullous keratopathy following cataract extraction (black) and graft failure (grey) with moderate-severe (grade IV) oedema.

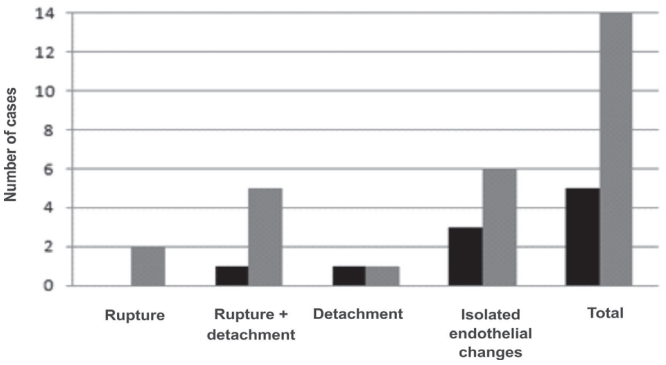
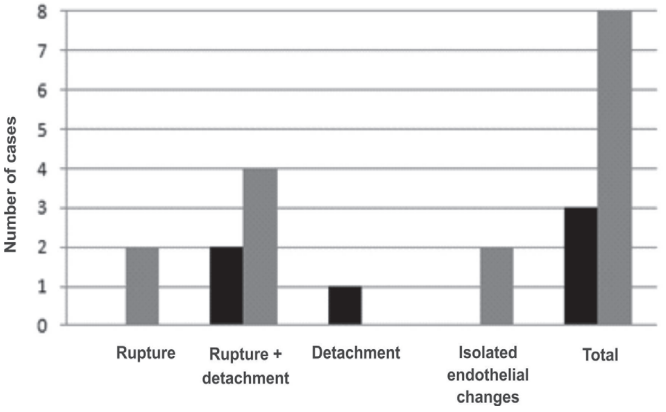


Chart 7

Histopathological changes found in cases of bullous keratopathy following cataract extraction (black) and graft failure (grey) with severe (grade V) oedema.



for examination, while samples from cataract extraction are only occasionally submitted. Most cases of both bullous keratopathy following cataract extraction and graft failure presented oedema grade I and IV, according to our classification.Therefore, no correlation was established between the diagnosis and the degree of oedema.

The main histopathological change found both in cases of bullous keratopathy following cataract extraction and graft failure was endothelial cell rarefaction. Since isolated endothelial changes were seen in only 17 of the 70 specimens (Chart 1), endothelial changes were frequently associated with changes to the integrity of Descemet's membrane — either isolated detachment, rupture, or both combined. We suggest that this association might not be due to chance alone — instead, changes to Descemet's membrane could be the cause of endothelial changes. Another explanation could be related to the way the pieces were sliced, a factor we had no control over due to the retrospective nature of this study. We are also aware that endothelial cells are easily altered by fixation and staining; therefore, it is difficult to obtain a precise image of their normal and pathological structures through microscopic examination of fixed, stained slides[3].Nonetheless, we underline the reliability

of endothelial histopathological findings based on studies such as William et al., who found a direct correlation between the number of endothelial cells in histological examination and the density of cells in specular microscopy[9].Our research, however, did not focus on the endothelium, as these changes have already been thoroughly studied. Instead, we focused mainly on Descemet's membrane, as it was altered in 53 out of the 70 cases (Chart 1 and Table 4), and because of the potential effects of oedema on the membrane and the possibility to institute early and effective treatment against such changes.

Since our main objective was to study the pathogeny of corneal oedema, we focused mainly on changes to the integrity of Descemet's membrane (Chart 1 and Table 4), which are mentioned in literature as possible causes of the condition[5].Among these changes, detachment was a frequent finding, either associated with rupture (40% of cases) or in isolation (17.14% of cases). Detachment was thus found in 57.14% of cases, which stresses the importance of recognising it as a treatable cause. However, it is still unclear whether the rupture could have strained Descemet's membrane to the point of detachment or whether the two events were concurrent. Among the changes unrelated to pathogeny, the most frequentwere folds, followed by thinning, thickening, distension, and bifurcations (Table 2).However, it is unclear whether these findings occurred as a consequence of oedema or by chance. Folds were roughly twice as frequent in cases of graft failure (42% of cases) than in bullous keratopathy following cataract extraction (20% of cases). This might be explained by the fact that folds tend to resolve over time on biomicroscopy, and cases of graft failure, particularly primary failure, usually undergo earlier transplantation.

Most studies addressing the aetiology of bullous keratopathy or corneal oedema, however, mention endothelial cell loss or dysfunction as the main aetiological factor[1,2,10,11].Few sources in the literature mention Descemet's membrane defects as a cause of corneal oedema[5], and they usually do not make reference to surgical trauma. Studies on changes to the integrity of Descemet's membrane after surgery usually focus on membrane detachment following cataract extraction and its treatment. Contrary to the literature, which limits the aetiopathogenesis of bullous keratopathy and corneal oedema mostly to endothelial changes or, on a smaller scale, solely to changes to the integrity of Descemet's membrane, our histopathological study has shown that changes to Descemet's membrane may be associated with endothelial changes in the pathogeny of bullous keratopathy following cataract extraction.

The suggestion that changes to the integrity of Descemet's membrane are probably the main pathogenic factor in cases of bullous keratopathy following cataract extraction could also be made for cases of graft failure. Despite the improving results of penetrating keratoplasty, graft failure still remains a significant problem. Common aetiologies include primary donor failure, surgical complications, complications related to intraocular lenses, persistent epithelial defects, rejection, infection, glaucoma, trauma, and relapse of primary corneal dystrophies[12].However, changes to endothelial cells are cited in literature as one of the main factors leading to corneal graft failure, as is the case for bullous keratopathy. Bell et al.observed a low initial endothelial cell count in sequential examinations by specular microscopy and a continuous decrease in cell density over the firstfive postoperative years in patients who met the criteria for late endothelial failure and who had been subjected to repeated keratoplasties[13].Santos et al., studying corneal specimens diagnosed as grafts failures, found endothelial decompensation as the most common histopathological finding[14].With the exception of studies such as Aurora et al., few papers mention Descemet's membrane defects as part of the aetiopathogenesis of corneal graft failure[15].

Considering Descemet's membrane defects — which, in our study, involved mostly detachment associated or not with rupture — as an important factor in the pathogeny of bullous keratopathy following cataract extraction and graft failure after keratoplasty, this change should always be investigated in cases of oedema occurring in the immediate postoperative period of these procedures. Descemet's membrane detachment is a rare but severe complication of intraocular procedures[16].It is not uncommon in cataract surgery, with a reported incidence of 0.5% and 2.6% in extracapsular surgery and phacoemulsification, respectively[17].Many approaches have been suggested for the condition, such as observation[18], injection of C3F8[19,20] or SF6[8,20,21] gas into the anterior chamber, injection of viscoelastic[22], transcorneal sutures[23], and corneal transplantation. Descemet's membrane detachment can be classified into two types: planar, when stromal separation is under 1 mm; and non-planar, when the separation is greater than 1 mm. Both types of can be further classified as either peripheral or peripheral with central involvement[7].Vastine et al.suggest that the small planar detachments should be managed with observation, since many cases resolve spontaneously. Large planar or non-planar detachment or scrolled detachment require surgical intervention[24].Kim et al.recommend treatment with intracameral gas injection in cases of a detached yet intact Descemet's membrane[8].

In sum, as mentioned above, multiple approaches exist for treating Descemet's membrane detachment. There are some alternative treatments to corneal transplantation that can lead to complete resolution of corneal oedema and prevent the need for transplantation. Although repositioning may take some time, the endothelium/detached Descemet's membrane complex may remain relatively healthy in the anterior chamber, with corneal transparency eventually resuming when the repositioning takes place[21].Once again, we stress the need to thoroughly investigate the presence of this change in cases of corneal oedema in the immediate postoperative period of cataract extraction or corneal transplantation, in an attempt to avoid keratoplasty through early diagnosis and alternative therapies. We are aware that it is not always possible to visualise Descemet's membrane with a slit lamp in cases of severe corneal oedema[16], and because more sophisticated diagnostic methods are not always easily available, we suggest considering the use of the aforementioned alternative routine methods in cases of early corneal oedema following traumatic anterior segment procedures in an attempt to treat the condition before the first or successive keratoplasties.

Conclusion

This study used histopathology findings to demonstrate that changes to the integrity of Descemet's membrane, which in this study consisted primarily of detachment associated or not with ruptures, are frequently found in cases of corneal oedema following anterior segment surgery, in particular cataract extraction and corneal transplantation. The high frequency of this finding suggests that it plays an important role in the pathogeny of the studied cases of corneal oedema.

Endothelial cell rarefaction was also a frequent finding, although mainly associated with Descemet's membrane defects. The acknowledgment that not only the endothelium, but also Descemet's membrane detachment may be important in the pathogeny of corneal oedema following anterior segment surgery should prompt ophthalmologists to carefully investigate this change in the postoperative period and institute early treatment in an attempt to prevent the need for keratoplasty.

As this was a retrospective descriptive study, we hope our findings will encouragefuture prospective studies that may confirm our conclusions.

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