

CLINICAL SCIENCE

Corneal calcification after amniotic membrane transplantation

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Background/aims: Amniotic membrane transplantation (AMT) has become well established as a treatment for chronic epithelial defects, conjunctival reconstruction, and partial limbal cell deficiency. The aim of this study was to describe cases of corneal calcification following AMT and to search for risk factors that might predispose to this unusual finding.

Methods: Details of 117 AMTs on 93 corneas of 91 patients with a follow up period of at least 1 month performed since 1999 were collected prospectively. In those with calcification clinical photographs were studied and the medical records retrospectively examined.

Results: 15 calcifications in 117 AMTs (12.8%) were identified, occurring 3–17 (median 6.1) weeks after AMT, during a follow up period of 4–151 (median 25) weeks. Overall epithelial healing rate was 83%. Calcification covered a surface area between 0.7–40.5 mm² maximum size with varied morphology. The primary diagnosis was diverse. Risk factors included the use of phosphate eye drops and pre-existing calcification in the operative or other eye. No patient with a "patch" AMT developed calcification.

Conclusions: Corneal calcification occurs after some cases of AMT. A common risk factor was the postoperative use of phosphate containing eye drops.

Corneal calcification is classified into two types: band keratopathy and calcareous degeneration. Band keratopathy affects only the superficial layers of the cornea—that is, the epithelium, Bowman's layer, and the anterior stroma, with clarity of the cornea immediately next to the limbus and with densest deposits in the palpebral fissure.¹ This condition occurs in chronic inflammatory disorders² and hypercalcaemic states such as renal failure.³ On the other hand, calcareous degeneration of the cornea is much rarer, also affecting the posterior corneal stroma. It is usually seen in association with neoplasms or phthisis, but has been described in patients with persistent epithelial defects and dry eye,^{4–6} AIDS patients,⁷ graft versus host disease,⁸ and in patients treated with topical retinoic acid⁹ and drops containing phosphate.¹⁰

Amniotic membranes are gaining widespread popularity in a number of serious ocular surface disorders,¹¹ including non-healing corneal ulcers and persistent epithelial defects,^{12–15} treatment of ocular burns,^{16–18} bullous keratopathy,¹⁹ limbal stem cell deficiency,^{20, 21} and repair after pterygium excision.^{22–24} So far most publications have been favourable with the exception of severe corneal burns¹⁸ and the treatment of leaking filtering blebs.^{25, 26} Descriptions of complications have been few including three case reports of postoperative hypopyon attributed to a presumed local immune reaction,^{27, 28} a case of postoperative bacterial corneal ulcer,¹⁹ pseudopterygium,¹⁹ and pyogenic granulomas after pterygium repair.²³ We have found no previous case reports of calcification after amniotic membrane transplantation (AMT) in the literature.

The purpose of this study was to describe several cases of corneal calcification after AMT and to search for risk factors that might predispose to this unusual finding.

PATIENTS AND METHODS

To date (August 2002) in the department of ophthalmology, Universität Erlangen-Nürnberg, Erlangen, Germany, we have performed 150 consecutive AMTs, which have been prospectively followed since June 1999. Of these, 117 amniotic mem-

brane grafts have been performed for corneal conditions on 93 eyes of 91 patients with at least 28 days' follow up visit. Detailed information on diagnosis, surgical procedures performed, and outcome have been recorded at least preoperatively, at 1 month, 3 months, and thereafter at 6 monthly intervals. Clinical photographs, in the form of slides, were taken of each patient before surgery and at each follow up. Information was retrieved on age, sex, affected eye, primary diagnosis, number of layers of amnion used, surgical technique, location of the ulcer from the centre of the cornea (classified as central, mid-peripheral, peripheral, or combination of these), follow up times and presence of corneal calcification for all patients receiving AMT to the cornea.

In addition, all clinical photographs were examined for the presence of corneal calcification. In affected patients further retrospective data were collected, using the medical records, including information on length of history before surgery, Schirmer test results, previous surgery on the affected eye, presence of calcium or infection before surgery, and serum calcium and creatinine levels. A complete record of medication used in the week before surgery and period between surgery and calcification was taken, with particular attention to topical medication containing phosphate, those containing vitamin A, and the use of autologous serum.²⁹ To determine the full ingredients of all topical medication the Rote Liste³⁰ was consulted. This is a list of pharmaceutical agents licensed in Germany and includes details of all ingredients including preservatives and buffering agents. The nearest UK equivalent is the ABPI compendium of datasheets.³¹

Photographs of affected patients were scanned with a Nikon Slide Scanner LS-2000, to a resolution of 1843 × 1207 pixels. Each photograph was examined for timing and position of the calcification in relation to the most recent AMT, and the morphology and density of the calcified areas. Morphology was classified as: "single focus" where one single area of calcification dominated, "multifocal" where several small areas of calcification were present, "speckled" where the calcification formed in one focal area but consisted of multiple focal spots. Density was classified for the purpose of this study

Table 1 Primary diagnosis of patients undergoing amniotic membrane grafting with and without postoperative calcification

Primary diagnosis	Number in non-calcified group	Number in calcified group	Total
Herpes simplex virus	21	4	25
Chemical burn	10	1	11
Bullous keratopathy	10		10
Rheumatoid disease	8		8
Neuropathic cornea	5		5
Corneal tattooing to blind eye	1		1
Non-healing defect after PKP	3		3
Ocular pemphigoid	3	1	4
Bacterial corneal ulcer	4		4
Atopic eczema	3		3
Limbal stem cell insufficiency	3		3
Thermal burn	2		2
Herpes zoster	2		2
Trauma	2		2
Systemic graft-versus-host disease		2	2
Floppy eyelid syndrome	1		1
Marginal ulcer	1		1
Contact lens related ulcer	1		1
Pterygium	1		1
Acanthamoeba keratitis		1	1
Fuchs-Stevens-Johnson's syndrome		1	1
Interstitial keratitis	1		1
Fungal ulcer	1		1
Total	83	10	93

PKP = penetrating keratoplasty.

as: 1, visible, but not obscuring iris detail, 2, obscuring iris detail, 3, formation of a white plaque. The relation between the foci of calcification and the AMT was classified as "centred" where the centre of the AMT coincided with the area of calcification and "overlapping" where there was some overlap between the calcification and the original AMT, but they did not share the same centre.

The surface area and maximum length in pixels of each calcified area were measured using AnalySIS software (version 3.0, Soft Imaging Systems GmbH, Münster, Germany). A magnification factor was created by photographing a millimetre scale using the same slit lamp at the same magnification as the clinical slides.

All surgery was performed using cryopreserved amniotic membrane taken from women delivered by elective caesarean section. Amnion was preserved as described by Tseng³³ and Kruse.³² The medium used for preservation contained 250 ml of sterile glycerine filtered to 0.45 µm, 250 ml Dulbecco's modified Eagle medium, 50 000 U penicillin, 50 mg streptomycin and 1.25 µg of amphotericin B. The preservation time was 14–203 (median 78) days. The risk of transmitted infection was ruled out by serology 3 and 6 months after delivery.

Three techniques were used to perform the surgery depending on the indication for the membrane.³³ These were the use of at least one inlay or "graft" AMT to a focal area of corneal pathology, the use of an overlay or "patch" to cover the whole cornea, and a "sandwich" method where the two other techniques are combined to create at least two layers. These methods are described elsewhere.³⁴

All patients received ofloxacin drops and a bandage contact lens at the completion of the procedure. The contact lens and sutures were left in place for 4 weeks unless they were found to be loose. Topical and systemic postoperative medication was adjusted according to the indication for AMT and condition of the patient.

Where calcification occurred repeat AMT was performed after removal of the calcium plaque by scraping the calcium off using a hockey blade followed by intraoperative topical use of edetic acid (EDTA) to remove, where possible, all remaining visible traces of calcium.

All results were analysed using SPSS 10 for Windows release no 10.0.7 (SPSS Inc, Chicago, USA).

RESULTS

Comparing cases of AMT with and without calcification postoperatively

Twelve (12.8%) eyes of 10 patients and 15 (12.8%) of 117 procedures overall were affected by postoperative calcification.

The follow up time was 4–151 (median 25) weeks. The age range was 17–91 (median 67) years; 53 right and 39 left eyes were treated. There were 64 men and 29 women. Overall success rate, defined as complete epithelialisation within 4 weeks, was 95/115 (83%) of all AMTs performed. There was no statistically significant difference in follow up time, age, or sex between those with and without postoperative calcification.

All patients were affected by a non-healing epithelial defect or corneal thinning, without perforation, the primary diagnoses are shown in Table 1. The list of diagnoses was too diverse for statistical analysis. All three eyes with systemic graft versus host disease, treated with AMT, developed postoperative calcification. The median number of layers used per operation was two, with one to five layers being used in groups with or without calcification. Fisher's exact test showed no difference in the number of eyes treated with two or more than two layers between the two groups ($p=0.758$). The technique used for surgery (whether "graft," "patch," or "sandwich") was not associated with calcification (repeated Fisher's exact test lowest value $p=0.761$). Location of the original membrane was not associated with calcification (repeated Fisher's exact tests lowest value $p=0.244$).

Patients affected by corneal calcification

Notes were available for 9/10 of the patients who suffered calcification post AMT. Characteristics of patients affected by postoperative calcification are shown in Table 2.

The chronicity of the complaint leading to an AMT was between 1 and 94 (median 4) months. No calcification was seen in any patient after only "patch" AMT. The calcification was first identified between 3 and 17 (median 6.1) weeks after AMT. Four eyes underwent six reoperations for calcification, of which three eyes again recalcified.

Table 2 Description of eyes affected by corneal calcification after amniotic membrane grafting

Patient	Eye	Primary diagnosis	Secondary diagnosis	Age	Sex	Length of FU (weeks)	Graft technique	No of AMT before Ca ²⁺	No of retreatments	Risk factors	Other previous surgery†	Latest status
1	L	HSV		78	F	30	2 layer S	0	0	P, A	PKP	Conjunctival flap to cover defect and calcium
2	L	Acanthamoeba	Secondary glaucoma	37	F	47	2 layer G	2	0	P	ECCE (no IOL)	Calcified cornea, comfortable in bandage contact lens
3	R	HSV	Neuroopathic cornea	51	M	32	3 layer S	0	2	D, P, A	2nd PKP 2nd with ECCE	HLA matched PKP Still clear at 12 weeks
4	L	Fuchs-Stevens-Johnsons syndrome	Symblepheron	68	F	64	2 layer G	0	0	CP, P, S		Calcified with no epithelial defect
5	R	HSV	Non-healing corneal defect in graft	75	F	61	2 layer S	0	0	CS, P, A	PKP ICCE with scleral fixated lens	Calcified cornea with punctate staining
6	L	HSV	Bacterial ulcer	47	F	27	5 layer G	0	1	P, A		Residual calcium, HLA matched PKP, still clear at 12 weeks
7	R	Systemic graft versus host disease	Absolute dry eye	34	M	15	2 layer G	0	0	CO, D, P	ECCE	Huge plaque of calcium. Died before further treatment
8	R	Pemphigoid	Symblepheron	85	M	33	3 layer S	0	2	CO, P, S		No recurrence of Ca ²⁺ at 6 weeks
8	L	Pemphigoid	Symblepheron	85	M	23	2 layer S	0	1	CO, CP, S		Epithelialized cornea, no recurrence of Ca ²⁺
9	L	Thermal burn to cornea	Non-healing defect in graft	45	F	32	2 layer S	0	0	CP, P	PKP	Calcification off axis, but epithelial defect has recurred elsewhere

AMT = amniotic membrane transplant, FU = follow up, HSV = herpes simplex virus, *graft technique S = sandwich (graft and patch), G = inlay graft, †previous other surgery; PKP = penetrating keratoplasty, ECCE = extracapsular cataract extraction, ICCE = intracapsular cataract extraction, IOL = intraocular lens, F = female, M = male.
 CP = calcium noted preoperatively, CO = calcium noted other eye, CS = raised serum calcium, D = dry eye, P = phosphate containing drop given postoperatively, A = vitamin A ointment given postoperatively, S = severe ocular surface disease (included ocular pemphigoid or Fuchs-Stevens-Johnson disease)

Known risk factors for calcification for each eye are shown in Table 2. Three eyes had calcification documented in the notes and on photographs before the first AMT. Two patients developed calcification in the other eye—in patient 7 without AMT, in patient 8 with AMT in the right eye, the left eye going on to develop calcification without AMT. One patient had a marginally raised calcium level at 5.3 (normal range 3.6–4.8) mmol/l, this patient also had a raised creatinine level at 1.51 (normal range 0.5–1.2) mg/dl. All other patients with calcified cornea had normal serum and creatinine levels on repeated testing. Two patients had severe dry eye defined as documentation of this diagnosis in the medical notes and a recorded Schirmer test of 2 mm or less. Three eyes of two patients had severe ocular surface disease in the form of ocular pemphigoid or Fuchs-Stevens-Johnson syndrome.

All eyes, except the first AMT on the left eye of patient 8, received drops containing phosphate between surgery and the onset of calcification. These drops all contained either sodium hydrogen phosphate or sodium dihydrogen phosphate as an additive. Patient 2 also received sodium dexamethasone dihydrogenphosphate drops between surgery and the onset of calcification. Three of 10 eyes were on drops containing phosphate preoperatively whereas 9/10 were receiving drops containing phosphate postoperatively. The right eye of patient 8 was receiving autologous serum therapy at the time of calcification of his first re-treatment with AMT. Four patients received ointment containing vitamin A before calcification occurred.

Seven of 13 surgeries complicated by calcification were in eyes with previous infection: one acanthamoeba, four viral, and two clinical bacterial keratitis (culture negative). Four of 10 eyes had undergone penetrating keratoplasty before AMT.

The morphology, size, timing, and location of calcification are described in Table 3. Thirteen lesions were in the form of large dense calcific plaques; 11/13 of the foci were centred on the latest AMT.

DISCUSSION

To our knowledge there are no cases of corneal calcification after amniotic membrane described in the literature. We have experienced this problem in 12.8% of eyes receiving amniotic membrane for a corneal indication. Azuara-Blanco *et al* present a case of a patient with a white plaque after AMT, which they attribute to ciprofloxacin therapy³⁵; none of our patients were treated with ciprofloxacin. Ciprofloxacin deposits would be expected to disperse with time after treatment ceased.

In 10 eyes of nine patients developing this problem, all except one (case 2), developed calcification after their first AMT to that eye. All calcifications occurred within 17 weeks of the AMT with 11/13 occurring within 8 weeks. In all cases the location of the calcification coincided at least in part with the location of the amniotic membrane. All these facts suggest that the calcification was associated with the AMT.

Our epithelialisation rate of 83% compares favourably with that reported in the literature of 76–100%.^{12–15 17 21}

Morphology

The morphology of the calcification was quite variable with six dense calcific plaques, two less dense localised areas, four multifocal areas, and one of “speckled” appearance. This might suggest that the pathology in these cases may not be identical. The range in size of lesions measured was quite wide. The smaller less dense lesions did not require treatment. Two eyes underwent documented reduction in the size of the focus of calcification, in one (patient 4) this was as a result of intensive lubricant drops as an inpatient, in the other (patient 6) a large superficial piece fell off between visits, this patient required repeat AMT as the lesion did not reduce further in size forming a central calcific plaque. As yet we have no

Table 3 Photographic appearance of calcified areas after each amniotic membrane transplant complicated by post-operative calcification

Patient	Operation no*	Time until calcification (weeks)	Min length (mm)	Min area (mm ²)	Max length (mm)	Max area (mm ²)	Time between measurements (weeks)	Morphology	Density†	Relation to AMT‡
1	1	17			2.1	0.9		Multi focal	2	Centred
2	3	4	1.4	1.3	3.9	8.6	14	Single	2	Centred
3	1	8	6.5	11.5	6.5	14	6	Single	3	Centred
	2	6	3.6	3.8	3.9	4.3	14	Speckled	2	Centred
	3	5			7.7	17.4		Single	3	Centred
4	1	4	3.4	7.2	5.2	16.2	5.9§	Multi focal	2	Centred
5	1	3	1.3	0.6	1.4	0.7	49	Single	2	Overlap
6	1	6	4.7	9.3	6.0	13.2	18§	Single	3	Centred
7	1	4			8.9	28.6		Single	3	Centred
8R	1	3	6.3	10.2	10.5	35.3	4.4	Single	3	Centred
	2	6			8.1	40.5		Single	3	Centred
8L	1	5			4.4	4.9		Multi focal	2	Centred
9	1	13	0.9	0.4	1.5	0.8	19	Multi focal	1	Overlap

*Sequence number of AMT performed. †Density: grade 1 = iris detail still visible, grade 2 = iris detail not visible, grade 3 = dense white plaque.

‡Relation to AMT: Centred = centred under AMT, overlap = at least part of calcified area under original AMT. Only one measurable photo of calcification available. §Superficial calcium became dislodged between photographs.

Min length = minimum recorded length of largest focus of calcification, Min area = minimum recorded total area of calcification, Max length = maximum recorded length of largest focus of calcification, Max area = maximum recorded total area of calcification.

pathological confirmation of the type and distribution of calcium in these cases. However, two patients have undergone subsequent corneal grafting, we would expect to analyse these and future samples for publication at a later date. We might expect calcium deposits to be intracellular if associated with hypercalcaemia and extracellular if associated with local disease.

Risk factors

We have attempted to look at previously described associations with corneal calcification. It is thought that calcium deposition occurs when there is a local increase in calcium concentration. This may be due to an increased tear calcium level, increased phosphate concentration, desiccation or an increased pH as may occur with a reduced oxygen supply. All patients had at least one risk factor for the development of calcification (Table 2), three eyes having two risk factors, and two eyes having three risk factors.

One patient had renal failure and high serum calcium levels previously, these have been associated with corneal calcification. However, in these cases the calcification was superficial and either entirely involving the limbus or of the band keratopathy type.³

Two patients had severe dry eye as diagnosed in the medical notes and confirmed by a Schirmer's test with anaesthetic of less than 2 mm, further grading of dry eye was not possible owing to the retrospective nature of this part of the study. Several cases of dry eye associated with corneal calcification have been described in the literature.^{4,9} Two of our cases (three eyes) had systemic graft versus host disease. All these eyes developed corneal calcification suggesting an association. Corneal calcification has been described in at least two cases in the literature with this condition,⁴ one of which responded well to AMT.⁸

Ocular surface disease

Several cases previously reported have been associated with chronic epithelial defect^{5,6,36}; this is the main indication for AMT for corneal conditions and it is therefore not surprising that all our cases had this condition. In addition, three eyes had a history of severe ocular surface disease—that is, two ocular pemphigoid and one Fuchs-Stevens-Johnson syndrome.

Topical therapy

The deposition of calcium can be associated with an increased concentration of phosphate. This has been found to be associ-

ated with the use of phosphate containing steroids in particular dexamethasone 21-diphosphate with or without β blockers.¹⁰ We have noted that a significant number of topical eye drops contain phosphate salts as an additive, including many preserved ocular lubricants, β blocker drops and antimicrobials. We were interested to know what proportion of the affected patients had been exposed to phosphate postoperatively. We found that 9/10 patients were taking at least one drop containing phosphate postoperatively compared to 3/10 in the preoperative period. This might suggest that this could be a contributing factor to the development of calcification, although the medication was commonly given to all patients who received an AMT in the postoperative period. Three of the products containing phosphate used in this study are marketed in the United Kingdom and Germany with the same additives: Xalatan (Pharmacia), Liquifilm (Allergan), and Efflumidex marketed as FML in the United Kingdom (Allergan). A further two of the medications used were not distributed by the same manufacturer, but had an equivalent in the United Kingdom that also contained phosphate: Timosine (Chibret) is similar to Timoptol (MSD), Artelac is similar to Isopto Plain (Alcon). Dexasine (Alcon) contains the salt dexamethasone phosphate in preparation marketed in Germany, the equivalent Maxidex (Alcon) marketed in the United Kingdom contains dexamethasone, this however is not described as the phosphate form³¹ though it does contain a phosphate additive. Three of the medications used in the study have no equivalent in the United Kingdom: Hylocomod (Ursapharm), Vidisept (Mann), and Corneregel fluid (Mann).

Four patients were using vitamin A ointment postoperatively (Regepithel). The topical use of retinoic acid, which is the acidic form of vitamin A, has been described in two patients with dry eye and corneal calcification.⁹

The presence of AMT has been found to increase tear film concentrations of ofloxacin in rabbit eye with and without denuded epithelium although there was no significant difference in the corneal concentrations achieved³⁷; this suggests higher than normal concentration of topical medications may be found after AMT.

Pre-existing calcification

Three patients had some degree of calcification before their first AMT. This was treated at the time of surgery. AMT has been successfully used for treatment of corneal calcification in the past⁸ although it seems likely that those with this condition have an inherent problem that will predispose to calcification in the future. This was also taken to be the case in

the two patients who developed calcification in the other eye during the follow up period. This would suggest that at least five of the 10 eyes had some predisposing risk factor to corneal calcification not related to the use of AMT.

Study limitations

This was a long term observational study of a wide range of patients receiving AMT. As such it was impossible to keep all postoperative treatment consistent. As this was not controlled we cannot make any definitive statements about the way therapy may have affected the eye's tendency to develop calcification.

A weakness of this study is that we do not have any evidence that the other cases treated with AMT in this unit did not also have some of the described risk factors for calcification.

This study is necessarily limited by using a retrospective technique to examine the patients with corneal calcification in more detail.

CONCLUSIONS

In conclusion, this study demonstrates that calcification occurs after some cases of AMT. The condition has a tendency to recur after re-treatment making management difficult. Systemic graft versus host disease was universally associated with postoperative calcification. All patients had at least one "risk factor" for corneal calcification. The most common of these was the presence of calcification in either eye preoperatively and the use of drops containing phosphate. A randomised controlled trial would be required to confirm that phosphate containing drops genuinely increase the tendency of the cornea to calcify after AMT.

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