Original Article

Efficacy of Autologous Serum in Fixing Conjunctival Autografts of Various Sizes in Different Types and Grades of Pterygium

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Abstract

Purpose: To evaluate the efficacy of autologous serum in fixing conjunctival autografts of various sizes in different grades and types of pterygium and to determine the largest successfully secured graft size.

Methods: This prospective interventional study comprised 151 eyes of 151 patients belonging to age group of 21 to 64 years with different grades and types of primary and recurrent pterygium that underwent excision with inferior conjunctivo - limbal autograft secured with autologous serum. The autografts were measured with calipers and were grouped by size into three categories: Group A, small (5 × 5 mm); Group B, medium (5–7 × 5 mm); and Group C, large (>7 × 5 mm). The adhesive fixation power of autologous serum for the various conjunctival autograft sizes was determined for each group using the following criteria: graft stability, cosmetic appearance and complications in the immediate (first week) and two-months postoperative follow-up visits. Descriptive statistical analysis was used to calculate the percentage frequency of the variables.

Results: The groups A, B, and C included 48%, 22%, and 30% of the autografts, respectively. Overall, 93.34% of the grafts were stable with good cosmetic appearance. However, subconjunctival hemorrhage (36%), graft edema (36%) and graft retraction (13.5%) were the most common complications. The largest successfully fixed graft was 14 × 5 mm in size.

Conclusion: Autologous serum is efficient in securing conjunctival autografts of various sizes with minimal complications and satisfactory results, including good cosmesis.

Keywords: Pterygium; Conjunctiva; Autograft; Fibrin Glue; Suture; Autologous; Serum

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INTRODUCTION

Pterygium is a wing-shaped triangular encroachment of vascularized granulation tissue covered by conjunctiva

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in the palpebral area. First described in 10,000 BC by Sushruta, it is a fairly common degenerative disease with 9.5% to 13% prevalence in India and is commonly seen in dry and sunny climates with increased exposure to UV radiation, particularly in rural areas.[1-3] Pterygium can develop into a dense fibrous tissue and may cause visual impairment by inducing astigmatism or covering the pupillary area.

As medical management is ineffective, surgical excision is usually the only option available. Cosmesis, foreign body sensation, recurrent inflammation, visual impairment, diplopia from motility restriction and difficulty in wearing contact lens are the main indications for surgery.[4-9]

Surgical techniques have evolved from the bare sclera technique, autorotation of conjunctival flap, usage of amniotic membrane graft, or conjunctival graft to the recently developed conjunctivo-limbal autograft, which is considered the current surgical gold standard.[4,8,10] However, the debate is now focused on the most effective method to attain maximal patient comfort and faster rehabilitation after the adherence of the conjunctival autograft to the bare sclera by using either suture, fibrin glue, or autologous serum. The autologous serum has been proposed to be equal or comparable to that of suture and fibrin glue techniques.[4,5,7,8]

The efficacy of autologous serum has never been assessed, particularly whether it is similar for grafts of all sizes. Hence, we undertook this study to quantify the largest graft size and compare the efficacy of autologous serum in fixing grafts of various sizes.

METHODS

This prospective interventional study, undertaken according to the principles of the Helsinki Declaration, included 151 eyes of 151 patients undergoing pterygium surgery. The subjects were 21–64 years in age and had both primary and recurrent pterygia of various grades. Informed consent was obtained from each patient.

Patients with infection, inflammation, coagulation factor deficiency and any other associated ocular pathology were excluded from the study. The preoperative ocular examination included: refraction and assessment of best corrected visual acuity, slit lamp bio-microscopy, baseline intraocular pressure, fundus examination and photographic documentation of the pterygium.

Pterygium was categorized into four grades based on its encroachment over the cornea.[11,12]

1. Grade I - Pterygium head extending up to the limbus.
2. Grade II - Pterygium head extending between the limbus and a point midway between the limbus and the pupillary margin.
3. Grade III- Pterygium head extending beyond the point midway between the limbus and the pupillary margin, but not crossing the pupillary margin.
4. Grade IV- Pterygium head crossing the pupillary margin.

A surgeon performed all the surgeries using the standard conjunctivo- limbal autograft.

The eye was anesthetized with topical proparacaine 0.5%, one drop at five-minute intervals and repeated three to four times. Under aseptic precautions, and after proper draping, the eyelids were separated with a speculum and subconjunctival, and sub-pterygium 0.5 ml lignocaine solution (Xylocaine 2%) was injected. The conjunctiva over the pterygium was incised at the limbus with an arcuate curved incision. In each case, the conjunctiva automatically retracted from the limbus to almost 5 mm after the incision. Gentle dissection was then carried out between the conjunctiva and the pterygium tissue with the help of curved Vanna's scissors. The pterygium tissue was separated to the maximal possible extent towards the medial canthus. It was then excised and reflected over the cornea. The reflected pterygium was gently scraped with a No. 15 blade and peeled off along with its head and neck to attain smooth keratectomy. The bare sclera defect was measured with Castroviejo calipers and subconjunctival xylocaine was infiltrated in the inferior conjunctiva at the limbus. A 0.5 mm, oversized, conjunctivo-limbal autograft without tenon's was then harvested with the patient fixating in the opposite gaze. The graft was customized according to the length of the defect with a fixed width of 5 mm (denoting the retracted incised conjunctiva).

For graft bed preparation, a thin blood film over the exposed sclera was deemed adequate. Minimal saline adrenaline irrigation and tamponade with a cotton-tipped applicator were used to control excess hemorrhage. In case of insufficient bleed, the episcleral vessel was punctured with a 26-gauge needle.

The graft was laid over the bare sclera ensuring limbus to limbus contact and edge to edge apposition with the help of forceps. This was followed by an average wait time of three to five minutes for adherence and hemostasis to occur. Care was taken so that no bare sclera was left exposed. The eye speculum was removed carefully and the eye was then patched for 24 hours. Any intraoperative complication was noted [Figure 1].

The following day, the eye patch was carefully and gently removed under strict asepsis and the eye was assessed under slit lamp for visual acuity, graft adherence and any complication (s). Postoperatively, the patient was prescribed topical ciprofloxacin (0.3%) and dexamethasone (0.1%) combination eye drop, four times daily for two weeks. This was followed by a tapering dose over the next four weeks, and carboxymethyl cellulose 0.5% eye drops four times daily for six weeks. A six-month cumulative follow-up (at postoperative day one, end of weeks one, three, five, eight, and the fourth

and sixth month) was done for every patient. However, for most patients, the follow-up duration lasted from six months to five years. At each postoperative visit, a thorough slit lamp examination was carried out to document recurrence, complications, or any other problem.

RESULTS

We studied 151 eyes of 151 patients with both primary and recurrent pterygium, of which 127 were primary and 24 were recurrent. In the 127 cases with primary pterygium, 109 were nasal pterygia, 12 were temporal, and six were double-headed pterygia, while all recurrent pterygia were nasal [Figure 2].

The presenting complaints of patients were visual impairment, chronic irritation, recurrent inflammation of the pterygium and unsightly looks. Cosmesis was a concern with most of the patients, followed by chronic irritation, visual impairment and recurrent inflammation. In several patients, there was more than one presenting complaint [Table 1].

The age and sex distribution were suggestive of the maximum number of patients belonging to the 35–50-year age group (65.35%), and the minimum in the 70–86-year age group (13.38%). Regarding gender distribution, 87 (57.61%) patients were female and 64 (42.38%) were male [Table 2]. Pterygium of all grades were observed; however, the majority (59.60%) were of grade 2.

There was no correlation between the pterygium grade and graft size. The grading of pterygium depends on the corneal encroachment by pterygium, while the graft size is determined by the length of the pterygium along the limbus (limbal involvement).

As we incised the conjunctiva near the limbus, the conjunctiva revealed 5 mm of retraction. Therefore, a 5-mm width at the limbus was fixed in all cases, while the length along the limbus varied with the type of pterygium.

Intraoperatively, graft size was found to vary between 5 × 5 mm to 14 × 5 mm. Based on this, we classified the grafts into small (5 × 5 mm), medium (5–7 mm × 5 mm), and large (>7 × 5 mm) grafts [Table 3 and Figure 3].

Table 1. Chief complaints at the time of presentation

Chief complaint	No of patients	Percentage
Cosmesis	90	60
Chronic irritation	69	46
Visual impairment	60	40
Recurrent inflammation	30	20

Table 2. Age and Sex distribution of patients as per the different groups

Age range (years)	35-50		50-70		70-86	
Gender distribution	Male	Female	Male	Female	Male	Female
Group						
A	12	23	8	19	4	6
B	9	13	4	5	1	1
C	15	11	9	6	2	3

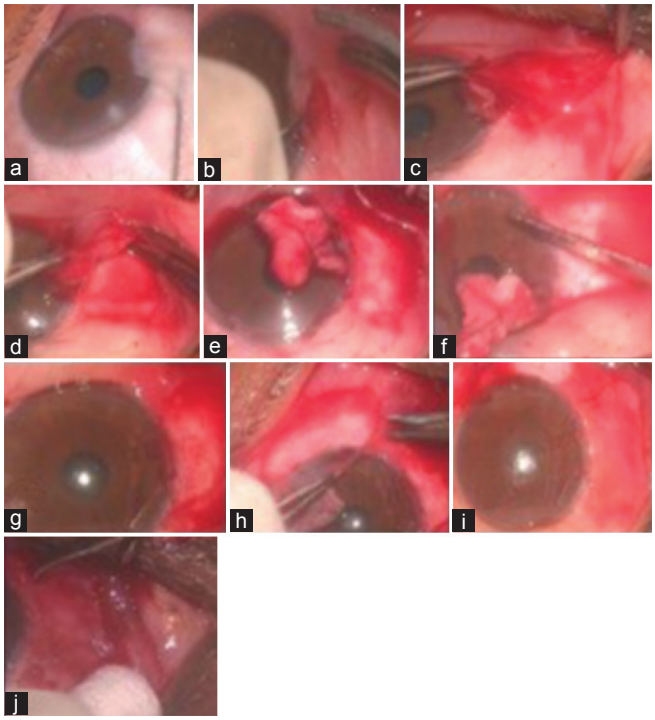

Figure 1. Surgical technique. (a) Subconjunctival xylocaine injection. (b) Conjunctival incision. (c) Pterygium dissection. (d) Cutting the pterygium. (e) Pterygium reflected on cornea. (f) Peeling from corneal surface. (g) Complete keratectomy. (h) Harvesting conjunctival graft. (i) Graft on sclera bed. (j) Edge to edge approximation.

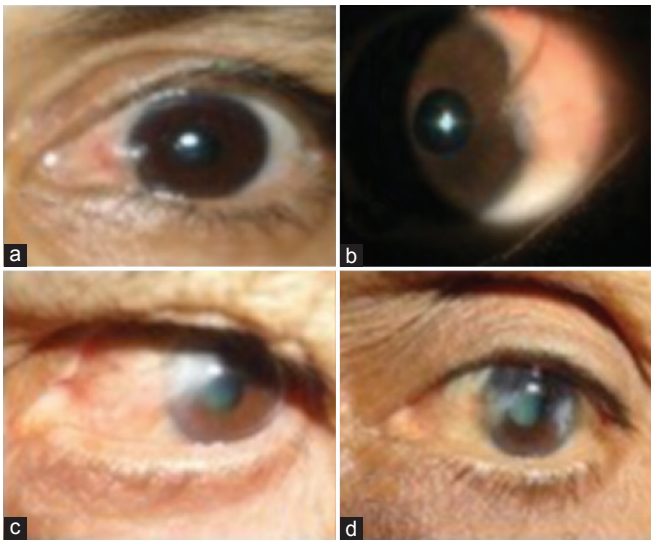

Figure 2. Preoperative. (a) Pterygium grade I. (b) Pterygium grade II. (c) Pterygium grade IV. (d) Double-headed pterygium.

Intraoperatively, there were no difficulties in fixing the graft. Postoperatively, complications noted in the immediate (first week) postoperative period were subconjunctival hemorrhage, edema, retraction and dislodgement of the graft.

The long-term follow-up duration was six months in 12 patients (7.94%) and six months to one year in 11 patients (7.28%). Most of the patients were followed-up for more than a year, with a duration of 1–3 years for 75 patients (49.66%) and 3–5 years for the remaining 53 patients (35.09%).

Delayed complications in the form of granuloma formation and recurrences were observed [Table 4 and Figure 4]. Further distribution of complications according to the graft size and age demonstrated higher immediate complications in the younger 35–50-year age group and no significant correlation of late complications with age. The frequency of complications increased with the size of the graft [Table 5].

Graft edema resolved by the second post-operative week in all the patients, while subconjunctival hemorrhage resolved by the third week in most patients. Of the nine patients with graft retraction on the nasal side, six patients had mild (<1 mm) retraction; hence, no intervention was performed. Suturing was required only in three patients where graft retraction was more than 1 mm, and it was performed with a 10-0 monofilament nylon suture. Only two sutures were sufficient to bridge the gap, and the sutures were removed after a week. All these nine patients had large grafts (>7 mm). No gap at the graft-host junction was observed at the end of six weeks in these patients. Graft dislodgement was seen in one elderly female who suffered from hemifacial spasm on the same side and this was managed by suturing the graft. None of the patients had graft infections.

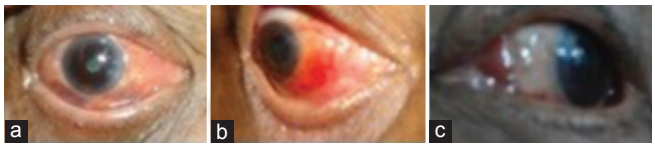

Figure 3. Graft size. (a) Small. (b) Medium. (c) Large.

Table 3. Distribution of patients according to different conjunctival graft sizes used in the study

Group A	Group B	Group C
Small grafts (5×5 mm)	Medium grafts (5-7×5 mm)	Large grafts (≥7×5 mm)
72	33	46

The late complications observed were graft infection, granuloma formation and recurrence. Granuloma formation at the graft host junction was seen in one patient of group C, and it was medically managed by topical prednisolone acetate eye drops. A recurrence of 1.32% was observed after two months of surgery in two cases of grade IV progressive pterygium where maximum graft size noted was 9 mm, and one case with fleshy recurrent pterygium of grade III with underlying fibrosis. The success of the procedure was defined as the absence of secondary suturing, and this was observed in 97.35% of cases.

On comparing complications in all three groups by the ANOVA test, the *P* value obtained was 0.795, which was not significant. This clearly showed that the complications noted in small, medium, and large grafts were comparable, and none of the groups showed a statistically significant difference in the complication rate over the other groups [Table 6].

We observed increased patient comfort and satisfaction as well as better cosmesis and reduced inflammation in the immediate post-operative period.

DISCUSSION

Cosmetic appearance is usually the main concern for patients with pterygium and the only remedy is surgical management. There are different emerging and ever-evolving techniques to achieve excellent cosmesis with minimal or no recurrence. Among all available procedures, pterygium excision with conjunctivo-limbal autografting realizes these objectives; thus, it is the currently preferred modality.[4,6,7,10] There are different techniques to fix this conjunctivo-limbal autograft, and they include the use of; sutures, fibrin glue and the newly emerged autologous serum.[4,6,13]

Suturing is the age-old-modality that is relatively easy to perform even for newly trained surgeons. Despite the technical ease and feasibility, the main disadvantage of this technique is the long duration of surgery and the postoperative discomfort due to increased

Table 4. Overall postoperative complications in different groups

Postoperative Complications	No. of patients	Group A	Group B	Group C	Total Percentage
Subconjunctival hemorrhage	24	6	11	7	16
Graft edema	24	10	8	6	16
Graft retraction	9	0	0	9	5.9
Graft dislodgement	1	0	0	1	0.66
Graft infection	0				0
Granuloma	1	0	0	1	0.66
Recurrence	3	0	1	2	1.32

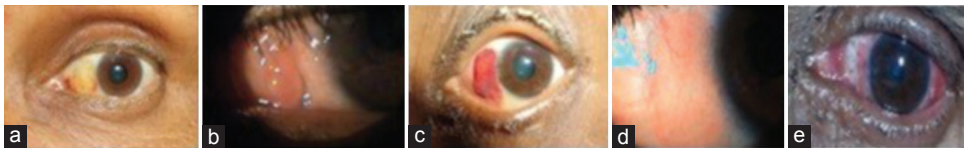

Figure 4. Postoperative complications. (a) Graft edema. (b) Granuloma. (c) Subconjunctival hemorrhage. (d) Mild graft retraction. (e) Marked graft retraction.

Table 5. Distribution of postoperative complications as per age and gender

Age range (years) / Gender distribution	Groups as per graft size	35-50		50-70		70-86		Total
		Male	Female	Male	Female	Male	Female	
Subconjunctival hemorrhage	A	2	1	1	1	0	1	24
	B	2	2	3	2	1	1	
	C	2	1	2	2	-	-	
Graft edema	A	5	2	2	1	-	-	24
	B	4	1	1	2	-	-	
	C	2	1	-	-	1	1	
Graft retraction	A	-	-	-	-	-	-	9
	B	-	-	-	-	-	-	
	C	3	2	2	1	-	1	
Graft dislodgement	A	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	
	C	-	-	-	-	-	1	
Graft infection		0	0	0	0	0	0	0
Granuloma	A	-	-	-	-	-	-	1
	B	-	-	-	-	-	-	
	C	1	-	-	-	-	-	
Recurrence	A	-	-	-	-	-	-	3
	B	-	1	-	-	-	-	
	C	1	-	1	-	-	-	

Table 6. Statistical summary for complications

	Result Details			
Source	**SS**	**df**	**MS**	***F*-ratio**
Between groups	8.4444	2	4.2222	F=0.23284
Within groups	272	15	18.1333	
Total	280.4444	17		

The *f*-ratio value is 0.23. The P=0.79. The result is not significant at P<0.05

inflammation and suture related complications. This may lead to prolonged healing, fibrosis and suture abscesses.[4,6] Suturing also has a higher recurrence rate when compared to fibrin glue, which is an alternative method of affixing the autograft to the bare sclera.[4,6-8] Also, fibrin glue has the advantage of better cosmesis and absence of suture related complications. However, because of the biological origin of fibrin glue, its use carries a risk of postoperative inflammation, anaphylaxis, and transmission of prions and parvovirus B19.[6,14,15]

The use of autologous fibrin glue is much safer, but it requires a good laboratory infrastructure and has a cumbersome, expensive and unacceptably long duration of production; thus, this method is not popular.[10,14] According to a study by Foroutan et al[14] if iodine preparations used for conjunctival disinfection before pterygium surgery are not properly washed off, they render fibrinogen compounds inactive.[16] This inactivation results in the loss of the adherence property of the glue, which might lead to improper and inadequate graft fixation.

Autologous serum forms a fibrin clot which adheres the conjunctivo-limbal autograft to the bare sclera. As this is the patient's serum and we are not using any foreign material, there is nothing to promote inflammation, and there is no issue of market scarcity [Figure 5]. Without inflammation, most of the suture and glue related complications are eliminated, and the subsequent chance of recurrence is lowered. Also, this method does not incur the additional cost of suture or glue. With all these advantages, we felt the need to evaluate the unprecedented benefits of autologous serum, for various kinds of pterygium. Therefore, we studied the efficacy of this novel technique in 151 eyes with different sizes, sites, grades and types of pterygium.

We treated 127 primary and 24 recurrent pterygium cases. Of these, 109 were nasal, 12 were temporal and six

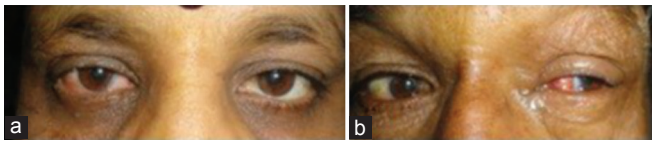

Figure 5. Comparison between operated eye and fellow eye, (a) Minimal lid edema and congestion noted with use of autologous serum. (b) Significant lid edema and congestion noted with suture surgery.

were double-headed. Two similar studies by Sharma et al and Shabaan et al on primary nasal pterygium included 80 and 50 eyes, respectively,[4,8] whereas, other studies had a mixed and smaller sample of primary and temporal pterygium ranging from 16 to 40.[5,7,17]

On comparing gender distribution, 87 (57.61%) eyes belonged to female patients and 64 (42.38%) to male patients; this female dominance was also observed by Sharma et al.[4] However, this was in contradiction to other studies and the universally accepted fact that pterygium occurs more commonly in the male population.[7-9]

On dividing our sample into various grades, 15.89% of eyes had grade I fleshy pterygium inducing significant astigmatism, recurrent inflammation, or unsightly look requiring intervention, 59.60% (or the majority) had grade II pterygium, 20.52% had grade III pterygium and 3.79% had grade IV pterygium. There was no significant correlation found with other studies as far as grading was concerned.[4,7,8]

The technique used by various surgeons have differed for the initial incision;[7,8,17,18] however, in our study, an extended subconjunctival removal of the pterygium tissue with reverse peeling was done with initial conjunctival cut at the limbus, with attachment of the slightly oversized [about 0.5 mm], thinnest possible, inferior conjunctival, Tenon's free graft.

We found out that thin grafts and meticulous dissection to remove the Tenon's layer with no residual sub-graft blood is of utmost importance to prevent the risk of graft retraction or rejection. It was noted that the surgeon's patience in dedicating adequate time during graft adherence was essential. Both parameters were considered in other studies as well, but surgeons took approximately 10 minutes on an average to ensure graft fixation.[8] On the other hand, we found that 3–5 minutes was sufficient to achieve similar results once we had optimally prepared the graft bed with a thin film of autologous serum and ensured appropriate edge to edge apposition of the thin graft after placing on the bare sclera.

Additionally, many studies took a conjunctivo-limbal graft from the superior quadrant,[10,17] but we took a graft from the inferior quadrant to leave the superior conjunctiva untouched for possible future surgeries. The division of grafts into groups (small, medium or large) based on their size was helpful to determine the efficacy of autologous serum to fix the largest conjunctival autograft. Rangu et al[17] reported an average graft size of 24 mm^2, but none of the earlier studies have considered graft size and compared differences and efficacy as we did. The largest size in our study was 14 mm × 5 mm, which is 70 mm^2.

It has been observed that patients who suffer from a coagulation factor deficiency or patients are regular users of aspirin or other blood thinners would not be good candidates for autologous serum fixation of the graft.[15] However, we encountered no such patient in our study.

Complications that were noted in the immediate postoperative period included graft edema in 16%, which was more than what other studies have reported (up to 10%). This can be explained by the fact that these other studies included cases with only primary pterygium, as opposed to our study where edema was more commonly seen in the eyes with recurrent pterygium (16/24 eyes, 10.59%). The lower incidence in primary cases (8/24 eyes, 5.29%) can be attributed to the minimal manipulation at the surgical site and the constant graft width of 5 mm in all cases. It spontaneously resolved with conservative treatment by the second postoperative week in all our patients, unlike other studies which reported a duration of six weeks for resolution.

On the first postoperative day, Sharma et al observed 32.5% eyes with subconjunctival hemorrhage. This is in contrast to the 16% seen in our study, a finding observed in grafts of all sizes, with the maximum incidence in group B where 11 eyes were involved. Less immediate subconjunctival hemorrhage was regarded as the excess blood which was frequently mopped from the graft site, and care was taken to ensure that only a thin film of serum remained to adhere to the graft. This completely resolved by three weeks in our study compared to six weeks in other studies.[8]

Tan et al discussed sub-conjunctival fibrosis and recommended the meticulous dissection of sub-epithelial graft tissue to prevent graft retraction.[19] Foroutan et al[14] reported that 20% of cases with graft retraction were managed conservatively without resuturing, while Shabaan et al[8] reported 12% and only one case required suturing. However, none of them mentioned any correlation with size. In our study, no retraction was seen with smaller-sized grafts, and no further gaping at the graft-host junction was noted at the end of six weeks in all three patients who underwent resuturing. Thus, the outcome was satisfactory.

Graft dislodgement that was seen in one elderly female (0.66%) can be attributed to the hemifacial spasm also associated with frequent eye rubbing.

All medium and small grafts were well apposed and taken up. As reported by Mitra et al, once the graft stays in place for the first 24 to 48 hours, it will stay adhered.[13] Prasanna Shankar et al[5] reported that 10%

of the grafts required suturing on the first postoperative day. According to Wit et al,[10] a suture-less and glue-free graft resulted in an even tension across the whole graft interface and no direct tension on the free edges. This result in a reduced stimulus for sub-conjunctival scar formation. Wit et al also suggested that the apposition of the eye lids to the bulbar conjunctiva provides a natural biological dressing, compression and a smooth, frictionless surface, which we also agree with.

The risk of recurrence is low if the procedure is properly performed. According to a study by Markovicha et al, the average duration for the appearance of complications, including recurrence after pterygium surgery is four to six months.[20] Recurrence of 1.32% was noted in the overall six-month follow-up in two cases of Group C and one case of Group B. It has been known that thick grafts not covering the limbus may increase the risk of recurrence. This was avoided in our study by ensuring thin grafts in all cases. In addition, our recurrence rate was significantly lower than that in other similar studies by Shabaan et al and Malik et al, who reported recurrences of 8% and 2.5% respectively, using similar suture-less and glue-free grafts.[8,7] The recurrence rate in our study was in accordance with the Massaoutis et al's criteria[17] which stated that the concept of surgical success in pterygium surgery can be defined as the provision of a white cosmetic conjunctiva, with no persistent symptoms and a low recurrence rate (less than 10%). A 'white cosmetic conjunctiva' which refers to excellent cosmesis, and an unnoticeable graft, was achieved in 84% of our patients, including large graft sizes [Figure 6]. A very low recurrence rate in our study can be attributed to the meticulous dissection of pterygium tissue, smooth keratectomy, and optimal bare site coverage by the graft.

As we have taken care to eliminate factors responsible for inflammation, patients achieved a good appearance approximately within six weeks. This was an additional advantage, as it addressed the most important issue that many patients initially presented, a finding that is in agreement with similar studies.[7,8]

Our criterion for a successful procedure, which was the absence of secondary suturing, was met in 100% of small and medium grafts and 91.3% of large grafts (overall 97.35%). We do agree that some complications were related to the graft size, but our findings concluded that 93.34% of overall grafts, including 78.26% of large grafts, were stable with good cosmesis. We would like to emphasize that the triad of adequate keratectomy, full coverage of the bare sclera with a thin graft and good control of inflammation is vital for satisfactorily securing even the largest conjunctival autografts, which was 14 × 5 mm in our study. Furthermore, the role of patient education in understanding the importance of compliance to medication, surgeon's instructions, and regular follow-up could further improve the outcome of this surgery.

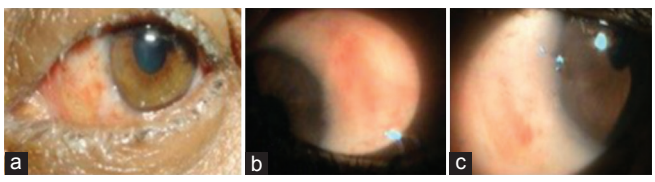

Figure 6. Post-operative follow-up findings. (a) 1st day post operation. (b) 7th day post operation. (c) 3rd week post operation.

Declaration of Patient Consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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