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Original Research Article

STUDY OF EPITHELIAL PHENOTYPE AFTER PTERYGIUM EXCISION BY USING CONJUNCTIVAL IMPRESSION CYTOLOGY.

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Abstract

Purpose: To compare the process of conjunctival epithelial regeneration after three types of pterygium excision procedures.

Methods: Twenty-two patients (27 Eyes) with primary progressive pterygium were randomly assigned to the bare-sclera procedure (group I, 9 eyes), pterygium excision with amniotic membrane transplantation (group II, 9 eyes), and pterygium excision with conjunctival autografting (group II, 9 eyes). Controls were healthy fellow eyes and ten eyes of age and sex matched subjects. Conjunctival impression cytology was performed with Millipore filter paper (0.025-0.22 microns) of 3 x 3 mm pieces pre and postoperatively at 2 weeks, 1, 3, 6, and 12 months. The nucleus to cytoplasm ratio of goblet epithelial cells and goblet cell density in pterygium area were calculated and compared between groups.

Results: Pterygium excision wounds healed in a similar four-stage process in all the groups, but at different rates. The nucleus-to-cytoplasm N/C ratio was highest at 2 weeks, 1 month, 3 months in group III, II, and I respectively, before gradually returning to control levels. Pre-operatively, the goblet cell density GCD in treated eyes was twice than controls (p 0.001) but fell to zero post-operatively. Goblet cells first appeared in group III, followed by group II. At 12 months, the mean GCD in group III was not significantly different from controls, whereas in group I and II were still less than controls (*P* 0.02)

Conclusion: Conjunctival autograft prevents pterygium recurrence more effectively than AMT. Impression cytology revealed a marked decrease in goblet cell density in the bare sclera and AMT cases while goblet cells returned to normal size, shape, and density in conjunctival autograft group. Even 1 year after surgery, the ocular surface remained abnormal with respect to epithelial phenotypes in all techniques.

Keywords: Bare sclera, Amniotic membrane transplantation (AMT), conjunctival autograft, Conjunctival impression cytology.

Introduction:

Impression cytology refers to the application of cellulose acetate filter material to the ocular surface to remove the superficial layers of the conjunctival epithelium.[1] Conjunctival impression cytology was introduced by Egbert *et al.*in 1977.[2] Morphology of the conjunctival epithelium is preserved, which permits the use of a limited range of histological techniques.[3] The purpose of this study was to compare the process of conjunctival epithelial regeneration after three types of pterygium excision procedures that are bare sclera, pterygium excision with amniotic membrane transplantation and pterygium excision with conjunctival autograft.

Material and methods:

This was a prospective, randomized, comparative case series study conducted in a tertiary care teaching institute where 27 eyes of 22 patients with primary progressive pterygium were studied. These eyes were randomly assigned to group I bare sclera (09 eyes), group II pterygium excision with amniotic membrane transplantation (09 eyes) and group III pterygium excision with conjunctival autograft (09 eyes). controls were healthy fellow eyes and ten eyes of age and sex matched subjects. Patients with other ocular surface disorders were excluded from the study. A written informed consent was taken from the patients after explaining the procedure. Ethical committee's approval was obtained before the study.

Patients underwent pterygium excision in the operation theater under peribulbar anesthesia in aseptic conditions. The lids were separated using eye speculum. The excision of pterygium was started using 15 no surgical blade and carried down till limbus. With the help of crescent blade, corneal surface was made smooth and regular.

In the bare sclera group (group I), excision of pterygium was carried out up to 4mm posterior to the limbus leaving sclera bare. Hemostasis was achieved with wet field-bipolar cautery. In the amniotic membrane group (group II) graft size was measured to cover entire bare sclera with Castroviejo caliper. The bare scleral area was covered with optimum size preserved wet amniotic membrane and was sutured with interrupted 10/0 nylon sutures. In the conjunctival autograft group (group III) after measuring the optimum size, a limbal-conjunctival graft was taken from super- temporal area of the same eye and transferred onto the bare sclera with limbal side opposing the limbus. The graft was secured with10-0 nylon sutures.

Postoperative treatment included steroid and antibiotic eye drops four times a day and ointment at twice daily. Patients were followed up at day 1, week 1 and 2, and 1, 3, 6, and 12 months postoperatively. At each visit, the complete ophthalmological examination was carried out to note any recurrence and complications. Recurrence was defined as a fibrovascular growth beyond the limbus onto the cornea up to 1 mm.

Conjunctival impression cytology was done pre-operatively and post-operatively at 2 weeks, 1, 3, 6, and 12 months. Conjunctival impression cytology was performed with Millipore filter paper (0.025-0.22 microns) of 3 x3 mm. The filter paper was removed with a peeling motion and then applied to a clean glass slide. The impression was transferred onto the glass slide by uniform gentle pressure. Fixing was done using 95% ethanol and 1% formalin. Specimens were subjected to periodic acid-Schiff (PAS) andpapanicolaou (PAP) stains. Samples were studied under the low and high power of compound microscope epithelial cell morphology, nuclear/cytoplasmic (N/C) ratio, and goblet cell density (GCD).

results were graded according to Nelson grading. [4] Statistical analysis was carried out with the Chi-square test.

Results: All patients had successful pterygium removal with no recurrence in the conjunctival autograft group while one

patient showed recurrence in AMT group, three patients in bare sclera group showed recurrence.

Pterygium excision wounds healed in a similar four-stage process in all the groups, but at different rates and with different final results. The nucleus-to-cytoplasm N/C ratio was highest at about 2 weeks in group III, at 1-month postoperatively in group II and at 3 months in group I, before gradually returning to control levels [Table 1].

Pre-operatively, the goblet cell density GCD in treated eyes was almost twice than that in control eyes (p 0.001) but fell to zero immediately post-operatively. Goblet cells first appeared (with the most rapidly increased density) in group III, followed by group II (which was four-fold less than pre-operative GCD). At 12 months, the mean GCD in group III was not significantly different from those in controls, whereas the mean GCD in group II and group I were still less than that of control (*P* 0.02) [Table 2].

Impression cytology study under a compound microscope revealed following findings. Controls showed normal conjunctival epithelium: Nelson grade-o with numerous plump Vocaloid goblet cells (arrow) with PAS +ve cytoplasm with periodic acid-Schiff stain in 27 (100%) eyes [Figure 1]. Group III showed highest N/C ratio with dysplastic epithelial cells with karyomegaly at two weeks [Figure 2]. Group II showed highest N/C ratio with dysplastic epithelial cells with karyomegaly at one-month post-operatively [Figure 3]. Group III showed numerous goblet cells with strongly PAS positive cytoplasm at 12 months post-operatively, but epithelial cell morphology still showed few dysplastic cells [Figure 4].

Group I showed non-goblet conjunctiva with dysplastic cells at 12 months post-operatively [Figure 5].

Table 1: Results of CIC in Study Cases and Control Group

CIC grade (Nelson)	Group I (n = 9)				Group II (n = 9)				Group III (n = 9)				Controls (n =27)
	2 wk	1 mth	3 mth	6 mth	2 wk	1 mth	3 mth	6 mth	2 wk	1 mth	3 mth	6 mth	
Grade 0 N/C 1:2	0	0	0	0	0	0	0	0	0	0	0	0	22
Grade 1 N/C 1:3	0	0	0	0	0	0	0	0	0	0	3	9	5
Grade 2 N/C 1:4-1:5	0	6	2	4	0	3	4	7	0	4	6	0	0
Grade 3 N/C 1:6	0	3	7	5	0	6	5	2	9	5	0	0	0

Table 2: Results of GCD in Study Cases and Control Group

GCD	Group I (n = 9)					Group II (n = 9)					Group III (n = 9)					Control (n =27)
	preop	1wk	1 mth	3 mth	12 mth	preop	1wk	1 mth	3 mth	12 mth	preop	1 wk	1 mth	3 mth	12 mth	
Absent	0	9	9	5	7	0	9	9	8	5	0	9	0	0	0	0
0-4/hpf	0	0	0	4	2	0	0	0	1	4	0	0	3	0	0	0
4-8/hpf	0	0	0	0	0	0	0	0	0	0	0	0	4	5	4	12
8-15/hpf	0	0	0	0	0	0	0	0	0	0	0	0	2	4	5	15
>15/hpf	9	0	0	0	0	9	0	0	0	0	9	0	0	0	0	0

Discussion:

Pterygium is a worldwide condition commonly seen in the Cameron pterygium belt located between 37-degree north and south of the equator. [5] Pterygium is a condition whose origin, development, and tendency to recur, all offer problems to ophthalmic surgeons. The recurrent pterygium is more aggressive in nature; therefore, it is also called as sleeping tiger. Surgical excision is the treatment of choice.

Simple excision of the pterygium alone has a very high rate of recurrence that is about 30-70%. [6] Various adjunctive strategies such as irradiation treatment, anti-metabolites, conjunctival autograft, limbal autograft, and amniotic membrane graft have been employed over the years to reduce the high recurrence rates with mixed success. [7, 8]

We studied the process of conjunctival epithelial regeneration after three types of pterygium excision procedures that bare sclera, amniotic membrane transplantation, and conjunctival autograft with the help of conjunctival impression cytology. Impression cytology provides a flat amount of an area as large as the size of the applied filter paper with well-preserved morphology. Conjunctival smears destroy much of the morphological information. [2, 3] Conjunctival biopsies provide information on a relatively small sample of the surface epithelium. [2, 3]

Conjunctival impression cytology is based purely on squamous epithelial and goblet cell abnormalities of exfoliated cells from conjunctival epithelium obtained by impression on Millipore filter paper and stained with PAP and PAS stain. The changes in epithelial cell morphology, nuclear/cytoplasmic (N/C) ratio and goblet cell density can be graded according to the scheme described by Nelson *et al*. According to this grading, grade 0 is (normal), in which epithelial cells are small and round, nuclei large with N/C ratio 1/2, goblet cells numerous, plump ovoid with intensely PAS +ve cytoplasm; Grade I is (slightly abnormal), in which epithelial cells are slightly longer and polygonal, nuclei small with N/C ratio 1/3, goblet cells lower in nos. PAS +ve cytoplasm; Grade II is(abnormal), in which epithelial cells are larger and polygonal, N/C ratio 1/4 - 1/5, goblet cells significantly lower in nos., less intensely PAS +ve cytoplasm.; Grade III is (significantly abnormal), in which epithelial cells are larger and polygonal, nuclei small andpyknotic, N/C ratio 1/6 with goblet cells

absent.[4] The nucleus-to-cytoplasm (N/C) ratio was first raised in group III(conjunctival autograft). Goblet cells first appeared (with the most rapidly increased density) in group III. The mean GCD in group III was not significantly different from those in controls, whereas the mean GCD in group I and group II was still
less than that of control. The surface epithelium morphology remained abnormal even at the end of 12 months postoperatively.
Limitations of this study: More number of samples needs to be studied to corroborate our findings.

Conclusion:
Conjunctival autograft prevents pterygium recurrence more effectively than AMT. Impression cytology revealed marked decrease in goblet cell density in bare sclera and amniotic membrane transplantation cases while goblet cells returned to normal size, shape, and density so also the staining characteristics conjunctival autograft group. Even 1 year after surgery, the ocular surface remained abnormal with respect to epithelial phenotypes in eyes treated by any of the two techniques.

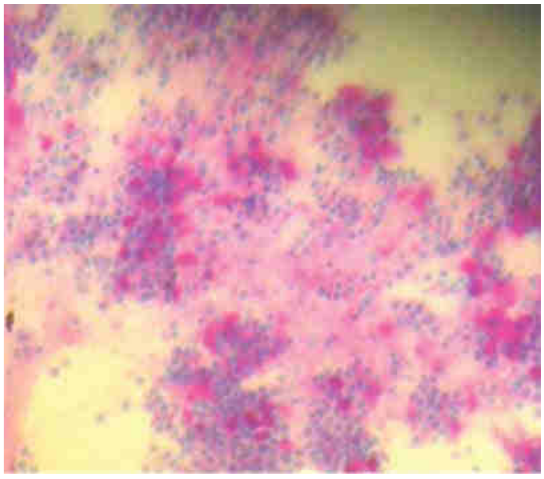

Figure 1: Low power of normal conjunctival epithelium: Nelson gr-o with numerous plump ovaloid goblet cells with PAS +ve cytoplasm, periodic acid-Schiff stain

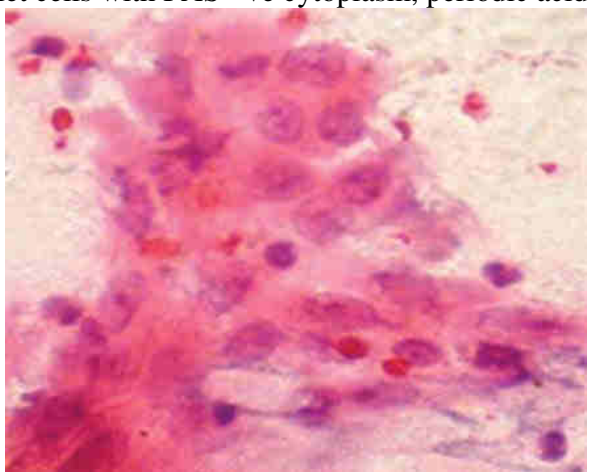

Figure 2: Group III-pterygium excision with counjunctivalautograft: Dysplastic epithelial cells with karyomegaly, some with an oval nucleus, nucleoli seen, pap stain, 400X. (two weeks post-operative)

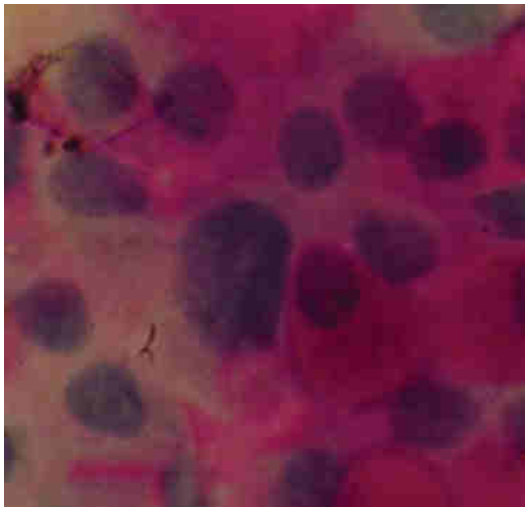

Figure 3: Group II-pterygium excision with AMT: a Dysplastic with cell with karyomegaly, with 2 PAS +ve goblet cells, oil immersion, 1000X, PAS stain. (one month post-operative)

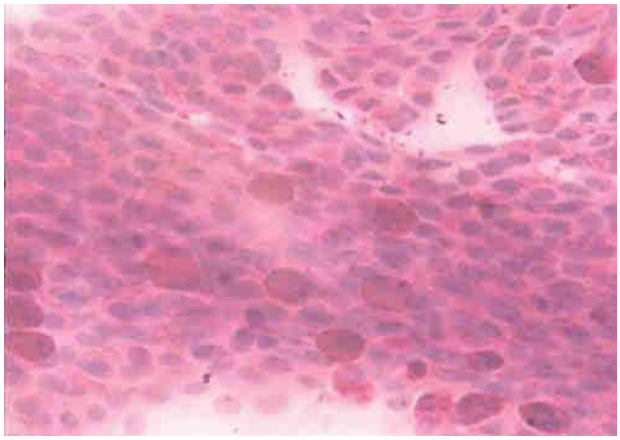

Figure 4: (Group III pterygium excision with counjuctival autograft), conjunctival squamous epithelium with few dysplastic cells, numerous *goblet cells* with strongly PAS +ve cytoplasm, 400X; PAS stain. (12 months post-operative)

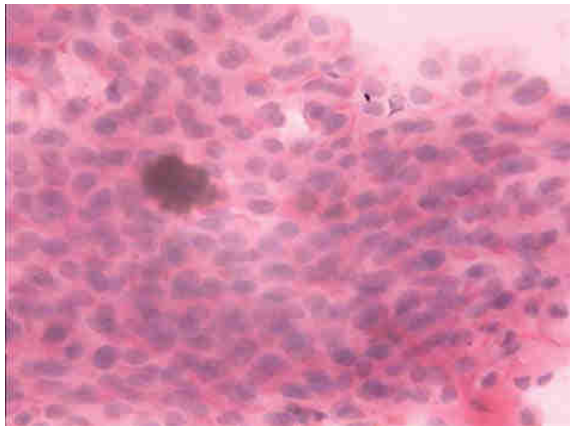

Figure 5: (Group I-pterygium excision with bare sclera), *non-goblet* conjunctival squamous epithelium with few dysplastic cells 400X; PAS stain (12 months post-operative)

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