



BOWMAN'S MEMBRANE TUCK-IN SURGERY- A MODERN APPROACH IN CASES OF NON-HEALING CORNEAL ULCER

Sunaina Hatila	Resident, Department of ophthalmology, SMS Medical College, Jaipur
Siddharth Maanju*	Resident, Department of Ophthalmology, SMS Medical College, Jaipur *Corresponding Author
M.K. Jharwal	Senior Professor, Department of ophthalmology, SMS Medical College, Jaipur
Dharamveer Singh Chaudhary	Associate professor, Department of Ophthalmology, SMS Medical College, Jaipur

ABSTRACT **Purpose:** To evaluate outcome of bowman's membrane tuck-in surgery in cases of post-infectious non-healing corneal ulcers. **Method:** A prospective interventional study of seventy (70) patients of corneal ulcers which were unable to heal despite adequate medical treatment after their infection were completely resolved, evaluated for the outcome of bowman's membrane tuck-in surgery. Corneal healing and best corrected visual acuity (BCVA) were assessed via fluorescein staining and Snellen's chart evaluated at 6 months post-operatively, respectively. Also, corneal contour were measured using topography and anterior-segment OCT. **Results:** Out of total seventy patients who remained in the study, 45 male and 25 female were included. Mean age of patient with standard deviation was 54.543 ± 15.454 years. The mean time of epithelisation was 2.614 ± 0.596 weeks. The mean size of ulcer along its maximum length and perpendicular to it were 2.514×2.621 ± 0.959×1.149 mm. Pre-operative mean BCVA was 0.106 ± 0.078 decimal units and post-operative mean BCVA was 0.271 ± 0.196 decimal units, showing a significant improvement with p value of p<0.0001 (S). Mean pre-operative value of corneal thickness was 214.272 ± 106.199 and the post-operative value was 416.628 ± 52.350 significantly better with p-value of p<0.0001 (S). Graft ectasia was noted in 11(15%) and corneal reinfection in 3 (4%) cases. **Conclusion:** Bowman's membrane tuck-in surgery significantly improves corneal thickness and BCVA after the period of 6 months in patients of post-infectious non-healing corneal ulcer with no special equipment and is cost-effective.

KEYWORDS : Bowman's membrane tuck-in surgery, non-healing, harvested, corneal thickness

INTRODUCTION

National programme for control of blindness was put forward with goal of turning down prevalence of blindness to 0.3% by year 2020.^[1] According to World Health Organisation, there are about 1.5-2 million new cases of corneal blindness worldwide annually^[2] with 25,000-30,000 cases in India alone^[3]. Fungi are of major concern being around 30-40% out of all, especially in country like India (varying topographically) and bacterial are second major concern.^[4] Among 6 layers of cornea, Bowman's membrane comes second and is acellular layer of 17 to 21 μm thick^[5] harbouring the sub epithelial nerve plexus. It is well known that bowman's layer do act as an obstacle for passage of micro-organism through the cornea into the eye^[6] and is evident that fibrous scar are formed at the focal losses of bowman's layer.^[7,8] It also have role in maintaining the structure and tectonic support of cornea as its privation can be seen in advance cases of keratoconus.^[9,10]

Corneal ulceration is a discontinuation of corneal epithelium may occur as a result of host cellular and immunologic response that involves the underlying stroma.^[11] Abusing agents can be bacteria, viruses, fungi or protozoan. Non-healing post-infectious corneal ulcers included in this study are those ulcers with completely resolved fungal and bacterial keratitis (i.e. smear and culture negative cases) but fail to heal in two weeks^[12,13] of time even after the infective agent was entirely gone away. Important causes of a non-healing corneal ulcer were persistent infection, neurotrophic keratopathy, distriachiasis, exposure keratopathy, severe dry eye, drug toxicity, steroid use.^[14] Ulcers if left untreated can progress sideways to form diffuse superficial corneal ulcers or can progress deeply resulting in perforation which can form adherent leucoma by plugging of iris tissue.

Every now and then various methods have been tried to combat the huge burden of corneal blindness on individual as well as community level. These are namely conjunctival flap surgery, use of glue like tissue adhesive, bandage contact lens, tarsorrhaphy, amniotic membrane grafting, autologous preparation and corneal as well lamellar grafting. Among them we are using lamellar grafting and more precisely bowman's layer grafting which seems to be more promising.

The above said methods were associated with disadvantages like the conjunctival grafts were associated with reduced visual ability and low cosmetic acceptance. Cyanoacrylate glue with bandage contact lens

cause severe foreign body sensation along with prevention of re-epithelisation, also the product cyanoacrylate is toxic to corneal endothelium and lens. Tarsorrhaphy cause cosmetic problem to the patients leading to patient dissatisfaction. Amniotic membrane is non-transparent, flaccid and difficult to handle causing it a less favourable approach for such pathologies. Autologous serum has risk of infections as the donor might be suffering from highly infective disease like hepatitis or can cause microbial infection due to lack of sterility. Corneal grafting has high chances of endothelial rejection and requires extensive use of topical and systemic steroids. In current scenario, most acceptable treatment for such corneal pathologies is corneal transplant in which demand exceeds the supply of donor tissue. With this advance technique of bowman's membrane tuck-in, we cross the above hurdles and deals major complications like tissue rejection^[15], early visual rehabilitation, control inflammation and restore innervations.^[16]

METHOD

A hospital based interventional prospective study was started after approval by ethical committee on March 2021 in a tertiary institute of India and the study obeys the tenets of Declaration of Helsinki. Total 76 patients (47 males and 29 females) of post-infectious non-healing corneal ulcers were recruited from March 2021 to December 2021. Among them 70 (45 males and 25 females) remained in study in follow-up of next 6 months. Patients fulfilling the criteria with corneal ulcer of maximum 4 mm in any dimension, reporting sterile corneal scrapping sample and with perforation not more than 2 mm were included in the study. After knowing the process, outcome failure, risk-benefit ratio, follow-up schedule and pre-operative investigations, consent was taken from the recruited patients through proper proforma. Patients having blind eye, infected corneal ulcers, corneal perforation >2mm, history of repeated graft failure, severe dry eye, Stevens-johnson syndrome, chronic illness like diabetes mellitus, AIDS patient, patients on immunosuppressant drugs, severe malnutrition, poor follow up patients were excluded from the study.

Outcomes were measured in terms of time taken for corneal epithelisation, best corrected visual acuity (BCVA) and corneal contour as determined by topography and Anterior Segment- OCT (AS-OCT).

SURGICAL TECHNIQUE

The therapeutic grade of cornea is selected and mounted in artificial

chamber (Katena, USA) and corneal epithelium is denuded with help of 15 number blade Bard Parker knife. Exposed tissue is stained with trypan blue dye. Then air is injected with the help of insulin syringe to create space between Bowman's membrane and anterior stroma, then sharp trephine is used to cut desired size. After making passage in between Bowman's layer and stroma with help of sharp crescent knife, then tissue is separated with blunt dissector like iris retractor. The desired size and shape is cut with corneoscleral scissor making it ready to be grafted. This tissue is kept slightly larger than the defect for adjusting in the stromal recess of recipient cornea.

After being anesthetised (peribulbar block) under aseptic conditions, base of ulcer is gently cleaned of debris with help of sharp crescent knife and anterior stromal recess is made. Grafts were set accordingly so that it does not reach to pupillary area and hinder any passage of light. Larger recess is made in perforated cases. Peritomy or cauterisation is done for any vascularisation or conjunctivisation. Bowman's membrane is then tucked-in and gently pressed to remove all the wrinkles and make the surface even. In few cases, limbal reconstruction was also done by taking limbal stem cells from healthy limbus and cut into small pieces to spread and secured with help of fibrin glue (Baxter, USA). Follow up of these patients were done on post operative day 1, week 1, 2, 4, 6 and monthly after 6 months. Post operatively we use, preservative free antibiotics (Moxifloxacin 0.5%) QID, tear substitute six times and short course of Tab Prednisolone 1 mg/per Kg body weight to control inflammation. Topical steroids (Prednisolone acetate 1%) were to be given only after epithelisation is over and then tapered off. On every visit, patients were checked for BCVA, slit lamp examination, photography, fluorescein staining, AS-OCT etc.

Statistical analysis

For the statistical analysis IBM SPSS software 22.0 version was used. Quantitative data were depicted as mean standard deviation while qualitative data illustrated in percentages. Paired-t test was used as a test of significance for comparing pre-operative and post-operative quantitative data. P-value of less than 0.05 was considered as significant.

RESULTS

In this study, the number of patients were 70 (n=70) with male exceeding the number of females. Mean age of patient with standard deviation was 54.543 ± 15.454 (in years). The mean time of epithelisation was 2.614 ± 0.596 (in weeks) suggestive of epithelisation time required to close the wound gap by granulation tissue. The mean size of ulcer along its maximum length and perpendicular to it were $2.514 \times 2.621 \pm 0.959 \times 1.149$ (in mm). 12 patients had perforation with iris tissue prolapsed through the defect resulting in formation of pseudocornea. These were documented as comorbidities.

In this study, pre-operative mean BCVA was 0.106 ± 0.078 decimal units and post-operative mean BCVA was 0.271 ± 0.196 decimal units, showing a significant improvement with p value of $p < 0.0001$ (S). BCVA improved significantly in almost all cases. Mean time taken for epithelisation was 2.614 ± 0.596 weeks. Mean pre-operative value of corneal thickness was 214.272 ± 106.199 and the post-operative value was 416.628 ± 52.350 (in μm) which is significantly better with $p < 0.0001$ (S).

DISCUSSION

In this study, corneal ulcers of resolved infection despite healing of fungal or bacterial keratitis were taken. Absence of infection confirmed firstly, by thorough slit lamp examination indicating lack of any purulent discharge or pus on the ulcer surface and absence of hypopyon. Secondly, by negative conjunctival smear and culture negative. The infection was resolved but extent of ulceration as a result of melting and inflammation ensue deep non-healing ulcer. The ulcers were large bowl shaped with some cases having deeper involvement causing perforation (as well as causing iris plugging) which respond drastically after Bowman's membrane tuck-in surgery. Graft ectasias were more common in such cases and noted in 11 (15%) and corneal reinfection in 3 (4%) cases in the form of hypopyon which was supposed to be reactionary. These were repositioned in another sitting and infection did subside with topical and systemic antibiotic within 2-3 weeks of time. In few cases, opacity was persistent.

Total number of patients initially taken for study was 76 out of which 2 were lost to follow-up due to COVID-19, 1 sustain trauma to the same

eye after surgery, 1 died because of road traffic accident and 2 didn't come for follow-up.

Dragnea DC et.al initially used Bowman's layer transplant as a treatment procedure for advanced keratoconus patients.^[17] This technique involves placement of Bowman's layer in mid-stromal pocket of affected cornea causing flattening and stabilization of future ectasia. Visual acuity was found to be improved but data not available. This study proved to be helpful in enlightening the importance of Bowman's membrane as a tissue.

BM lenticule stayed for longer time as compared to amniotic membrane and this factor perhaps played a major role in nerve regeneration, as studied corneal nerves take 6 months to regenerate. Also the normal nerve plexus is formed in Bowman's membrane^[18] for which it provide the microenvironment.

Dr Dharamveer Singh Choudhary et.al studied 17 patients of non-healing corneal ulcer with 10 female and 7 male.^[19] The mean age with standard deviation was 52.705 ± 16.513 years which was almost similar to our study. Mean epithelization time in weeks with standard deviation was 3.470 ± 0.977 which was slightly longer than our study as the size of ulcer taken by them was larger than ours ($3.4 \times 3.3 \pm 0.59$ along their longest axis and the axis perpendicular to it in mm). BCVA was significantly improved. This study is comparable to our present study.

As evident, BM lenticule provide greater support for epithelisation and adequate space for granulation tissue formation, associated with reduced activity of fibroblast. Also reduces corneal opacity with greater improvement of corneal width as noted after granulation tissue formation.

In all the cases of BM tuck-in, it was observed that it controlled the inflammation.^[19] The reason was due to its trophic effect. Inflammation control is very helpful in maintaining the transparency of cornea and to improve the visual potential, it also decreases the pain.

CONCLUSION

In today's scenario, there are a number of approaches available to provide results for non-healing ulcers, which are merely considered palliative rather than definitive as they often required more than one additional procedure. Our novel technique i.e. BM lenticule tuck-in surgery has more promising results as it provide support to the thinning tissue by covering the defect produced by ulcer (BM being an innate membrane that replenish the cornea along with facilitating epithelisation).

The ease of surgery, the smaller and simpler post operative regime was also responsible for its high acceptance for surgeon as well as patients. Also, the procedure being quiet simple (although harvesting BM lenticule is moderately tricky), its small learning curve, handful of instruments, minimum resources and more eminently the use of therapeutic grade of cornea for extracting BM lenticule lead to a thrilling victory.

FINANCIAL SUPPORT AND SPONSERSHIP: Nil

CONFLICTS OF INTEREST: Nil

Table 1: Comparison of pre-operative BCVA and post-operative BCVA

	Mean	Std Dev	SEM*
Pre operative BCVA (decimals)	0.106	0.078	0.025
Post operative BCVA(3 Months) (decimals)	0.271	0.196	
Difference	0.165		
95% CI	0.1151 – 0.2149		
Result (p value)	0.0001		

*Standard error of the mean

Comparison of pre-operative corneal Thickness v/s post-operative corneal thickness (μm)

	Mean	Std Dev	SEM ^o
Pre operative corneal thickness(μm)	214.272	106.199	14.152

Post operative corneal thickness(μm)	416.628	52.350	
Difference	202.356		
95% CI	174.374 – 230.338		
Result (p value)	0.0001		

◦Standard error of the mean

KEYWORDS

AS-OCT: anterior segment ocular coherence tomography

BCVA: best corrected visual acuity

BM: bowman's membrane

REFERENCES

1. NPCBVI: Statistics available from <https://nhm.karnataka.gov.in>
2. Insan, N.G., Mane, V.P., Chaudhary, B., Danu, M.S., Yadav, A., & Srivastava, V. (2013). A review of fungal keratitis: etiology and laboratory diagnosis.
3. NPCB: Statistics Available from <http://www.npcb.nic.in>
4. Leck, A. K., Thomas, P. A., Hagan, M., Kaliamurthy, J., Ackuaku, E., John, M., Newman, M. J., Codjoe, F. S., Opintan, J. A., Kalavathy, C. M., Essuman, V., Jesudasan, C. A., & Johnson, G. J. (2002). Aetiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis. *The British journal of ophthalmology*, 86(11), 1211–1215. <https://doi.org/10.1136/bjo.86.11.1211>
5. Tao A, Wang J, Chen Q, Shen M, Lu F, Dubovy SR, Shousha MA. Topographic thickness of Bowman's layer determined by ultra-high resolution spectral domain-optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011 Jun 1;52(6):3901-7.
6. KAYES, J., & HOLMBERG, A. (1960). The fine structure of Bowman's layer and the basement membrane of the corneal epithelium. *American journal of ophthalmology*, 50, 1013–1021. [https://doi.org/10.1016/0002-9394\(60\)90354-8](https://doi.org/10.1016/0002-9394(60)90354-8)
7. Moller-Pedersen, T., Cavanagh, H. D., Petroll, W. M., & Jester, J. V. (2000). Stromal wound healing explains refractive instability and haze development after photorefractive keratectomy: a 1-year confocal microscopic study. *Ophthalmology*, 107(7), 1235–1245. [https://doi.org/10.1016/s0161-6420\(00\)00142-1](https://doi.org/10.1016/s0161-6420(00)00142-1)
8. Lagali, N., Germundsson, J., & Fagerholm, P. (2009). The role of Bowman's layer in corneal regeneration after phototherapeutic keratectomy: a prospective study using in vivo confocal microscopy. *Investigative ophthalmology & visual science*, 50(9), 4192–4198. <https://doi.org/10.1167/iovs.09-3781>
9. Sykakis, E., Carley, F., Irion, L., Denton, J., & Hillarby, M. C. (2012). An in depth analysis of histopathological characteristics found in keratoconus. *Pathology*, 44(3), 234–239. <https://doi.org/10.1097/PAT.0b013e3283511b42>
10. Zimmermann, D. R., Fischer, R. W., Winterhalter, K. H., Witmer, R., & Vaughan, L. (1988). Comparative studies of collagens in normal and keratoconus corneas. *Experimental eye research*, 46(3), 431–442. [https://doi.org/10.1016/s0014-4835\(88\)80031-9](https://doi.org/10.1016/s0014-4835(88)80031-9)
11. Byrd, L. B., & Martin, N. (2021). *Corneal Ulcer*. In StatPearls. StatPearls Publishing.
12. Mohan, S., Budhiraja, I., Saxena, A., Khan, P., & Sachan, S. K. (2014). Role of multilayered amniotic membrane transplantation for the treatment of resistant corneal ulcers in North India. *International ophthalmology*, 34(3), 485–491. <https://doi.org/10.1007/s10792-013-9834-3>
13. Versura, P., Giannaccare, G., Pellegrini, M., Sebastiani, S., & Campos, E. C. (2018). Neurotrophic keratitis: current challenges and future prospects. *Eye and brain*, 10, 37–45. <https://doi.org/10.2147/EB.S117261>
14. Lee, H. K., Ryu, I. H., Seo, K. Y., Hong, S., Kim, H. C., & Kim, E. K. (2006). Topical 0.1% prednisolone lowers nerve growth factor expression in keratoconjunctivitis sicca patients. *Ophthalmology*, 113(2), 198–205. <https://doi.org/10.1016/j.ophtha.2005.09.033>
15. Parker, J. S., Dockery, P. W., & Melles, G. (2020). Bowman Layer Transplantation-A Review. *Asia-Pacific journal of ophthalmology (Philadelphia, Pa.)*, 9(6), 565–570. <https://doi.org/10.1097/APO.0000000000000336>
16. Lagali, N., Germundsson, J., & Fagerholm, P. (2009). The role of Bowman's layer in corneal regeneration after phototherapeutic keratectomy: a prospective study using in vivo confocal microscopy. *Investigative ophthalmology & visual science*, 50(9), 4192–4198. <https://doi.org/10.1167/iovs.09-3781>
17. Yang, A. Y., Chow, J., & Liu, J. (2018). Corneal Innervation and Sensation: The Eye and Beyond. *The Yale journal of biology and medicine*, 91(1), 13–21.
18. Ting, D., Henein, C., Said, D. G., & Dua, H. S. (2021). Amniotic membrane transplantation for infectious keratitis: a systematic review and meta-analysis. *Scientific reports*, 11(1), 13007. <https://doi.org/10.1038/s41598-021-92366-x>
19. BOWMAN'S MEMBRANE LENTICULE TUCK-IN: A NOVEL CONCEPTUALIZATION FOR THE MANAGEMENT OF POST INFECTIOUS CORNEAL ULCERS AND PERFORATION, Dr Dharamveer Singh Choudhary, Dr Pratibha Choudhary, Dr Siddharth Singh Maanju, Dr Gargi Verma, Prof. Kishor Kumar, Dr Anamika Choudhary INDIAN JOURNAL OF APPLIED RESEARCH: Volume-11|Issue-7|July-2021.