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Efficacy of Postoperative Systematic Immunosuppression with Methotrexate in High Risk Penetrating Keratoplasty

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Abstract

Aim: To compare the outcome of high risk penetrating Keratoplasties in patients on postoperative oral methotrexate with patients on standard regimen.

Design: Retrospective comparative case series.

Materials and Methods: Data was obtained by reviewing case records of high risk optical Penetrating Keratoplasty (PK) patients operated between Jan 2016 to Dec 2018 at our institute (P.B.M.A.'s H.V. Desai Eye Hospital) and who followed up for at least six months. Pediatric, therapeutic, tectonic and lamellar Keratoplasties were excluded. High risk cases had one or more of the following criterias: 1) previous failed grafts 2) presence of >2 quadrant stromal vascularization 3) unstable ocular surface 4) disorganized anterior segment 5) total corneal scars with no view of anterior chamber. Of the total 33 cases, 21 receive oral methotrexate whereas 12 cases were in the control group.

Results: The mean follow up was 73.14 weeks in methotrexate group and 71.33 in control group. There were totally 13 clear grafts (Clarity- Grade 4-3) in methotrexate group (61.9%) and only 4 in the control group (33.33 %). Patients on systemic Methotrexate were found to have a statistically significant chance of having a grade 4 clear graft as compared to the control group. (Two sided test P value=0.0374). It was also found that patients with multiple high risk factors on systemic Methotrexate were found to have a statistically significant chance of having grade 4 clear grafts as compared to the control group. (Two sided test P value=0.0197).

Conclusion: Systemic immunosuppression with oral methotrexate has shown some promising results in the postoperative management of high risk P.K.s in our series. The efficacy, safety and cost of this drug makes it a very viable option especially in patients with multiple high risk factors who form the major chunk of all cases of P.K.s done in the developing countries.

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Introduction

Traditionally corneal transplant is said to be the most successful amongst solid organ transplants [1] as it is an immunologically privileged tissue, due to lack of blood vessels and lymphatics. However this is a luxury available to the corneal surgeon after penetrating Keratoplasty (PK) in quiet eyes with inactive corneal scars and in non-vascularized recipient beds. This advantage is lost in situations which can be considered as high risk Keratoplasty [2] where multiple grafts, dis-organized anterior chamber, vascularized corneal opacity, ongoing inflammatory-infective process or a combination of the above exists. To raise the stakes, often this is the only treatable eye of the patient.

Systemic immune suppression is indicated in such situations, as with other organ transplants [3]. Efficacy, long term systemic safety and cost to the patient have to be borne in mind while prescribing and continuing such medication. In our series we have sought to compare graft clarity in patients on immune suppression with Methotrexate and those not receiving any immune suppression in high risk grafts. Methotrexate, a Folic acid antagonist has been used extensively in systemic immune suppression for decades. It has also been used in a variety of autoimmune ocular conditions. It is known to have a good safety profile and in the context of our patient population is affordable.

Materials and Methods

This is a retrospective comparative case series conducted at a tertiary level eye center in a developing country, run by an ophthalmic charity. Which It evaluates the efficacy of systemic Methotrexate in ensuring graft clarity and reducing the incidence and severity of immunological rejection in high risk penetrating Keratoplasty (PK). Data was obtained by reviewing case records of high risk optical P.K.s operated between January 2016 to December 2018 (at PBMA's H V Desai

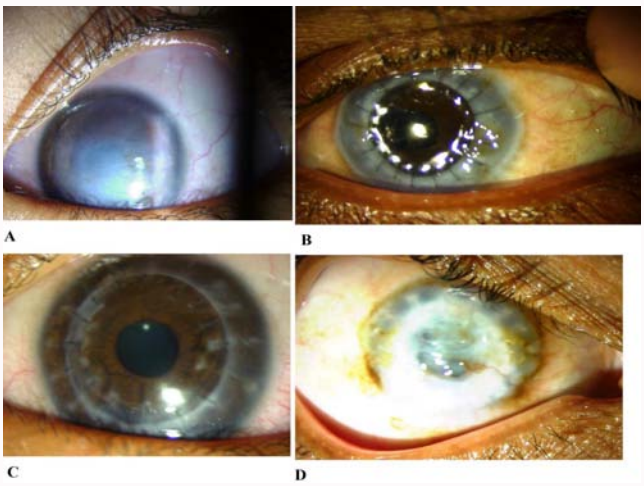

Figure 1: A: HSV scar with deep vascularisation. **B:** Postop on Methotrexate. **C:** Postop (HSV). **D:** Preop. twice failed P.K.

Eye Hospital, Pune, India), and who followed up for at least for a year. Patients who developed a completely opaque graft were treated as failures and not expected to follow up or continue with the treatment.

The cases were divided into a study treated arm who received postoperative oral methotrexate and a control arm that were given standard regimen of topical steroids and short course of systemic steroids postoperatively. All surgeries were done by one experienced corneal Surgeon (SJ). Exclusion criteria were pediatric cases, therapeutic, tectonic, lamellar grafts and patients a following a follow up of less than a year.

Cases included in the control arm were operated between 2014-2015, when systemic immunosuppression was not practiced as a standard protocol, at our centre. From 2016 systemic immuno suppression was the revised protocol for high risk cases, while the surgical technique remained the same. Hence, a selection bias of assigning patients less at risk, to the control arm would have affected outcomes, if taken from this period.

A high risk case was defined as [2,4] - 1) Previous failed grafts 2) Presence of >2 quadrants stromal vascularization 3) Unstable ocular surface with repeated epithelial breakdown, inflammation e.g. post chemical injuries 4) Post Herpes (HSV keratitis) cases with deep vessels and 5) Cases with disorganized anterior segment due to peripheral anterior Synaechiae, large adherent leucomas or cases with no view of anterior chamber. A note was made of the total number of high risk factors (Figure 1).

For all eye donation calls, in situ corneoscleral rim excision was done by our eye bank staff and donor tissue was collected in M.K. Medium under aseptic precautions. Donor corneas were evaluated by slit lamp microscopy and specular microscopy (Konan). Tissue was labelled as optical grade if following criteria were fulfilled- [5] 1) exposure keratitis <30% to 40% 2) stromal haze or oedema of mild to moderate degree 3) Central DM folds <5 and 4) endothelial cell density of 2000 & above.

In all cases donor grafts were oversized by 0.5 mm than recipient. Commonly used graft sizes were 8.0 mm × 7.5 mm and 7.5 mm × 7 mm. Surgeries done were P.K. alone, P.K. triple (with cataract extraction & IOL) and P.K. with additional surgeries like anterior vitrectomy, IOL explant, pupilloplasty etc. Interrupted suturing with 10/0 nylon was done in all cases.

Postoperatively, patients in the control arm received oral prednisolone for two weeks in tapering doses. In the treatment arm, oral methotrexate 7.5 mg OD once in a week along with folic acid 10 mg OD five times a week was given for one year was given.

Baseline Hemogram (Hemoglobin & complete blood count), Liver function Tests (Serum Bilirubin, SGOT, SGPT, Serum Alkaline Phosphatase), BUN, Serum Creatinine, Chest X-ray and ECG was done before starting oral methotrexate. Blood investigations were repeated every two weeks for first 6 weeks and then monthly to watch out for any side effects [6]. Topically both groups received the same treatment, i.e., antibiotics, artificial tears, antiglaucoma drugs (if needed), and topical steroids, which were continued for one year in tapering doses.

Graft rejection was defined as presence of one or more of these signs [7]; Mild if there was localized epithelial oedema, with increased stromal thickness but no aqueous cells and [1-5] Keratic Precipitates (KP); Severe rejection if diffuse graft oedema was present with >5 K.P.s, inflammatory cells in the stroma (not due to infection), endothelial rejection line or increased stromal thickness with aqueous cells.

Graft clarity was graded as [7] Clear grafts (Grade 4) if they were optically clear with excellent view of iris details,

Borderline/Grade 2-3 if there was moderate to significant haze with or without good view of iris details, and Failed (Grade 0-1) for opaque grafts with poor view of iris and anterior segment details.

A note was also made of distribution of eyes in the two groups with recurrent corneal epithelial defect two months after surgery which could be considered suggestive of an unstable ocular surface, a clinical etiology of HSV, disorganized anterior segment or secondary glaucoma.

All the patients had seen by the operating consultant and a blinded senior fellow for interpreting the graft rejection and graft failure as per standard guidelines. Inter-observer variation had been worked out between the two for another clinical trial and was kappa=0.92.

Grade 4 graft clarity was the primary outcome measure of the study. Statistics was performed using the stata software and non-parametric Wilcoxon Rank sum test was used to validate the difference in outcome between the two groups and also to analyze

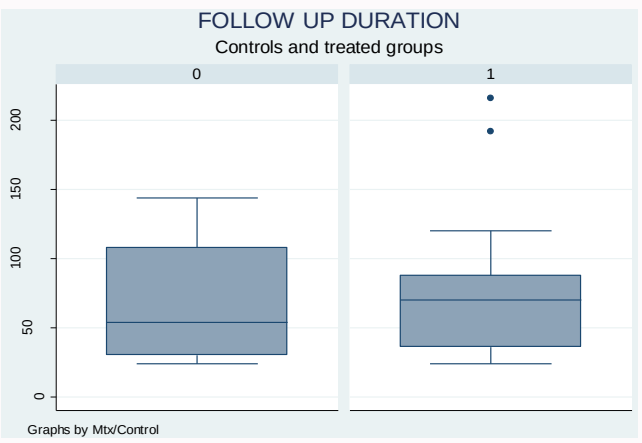

Figure 2: Follow up Duration.

whether any confounding factors would be operative between the two groups. Non parametric tests are good for a small data set and do not require a normal data distribution.

Results

Patients were distributed as controls who did not receive any systemic immune suppression [8-12] with a mean age of 45.17, 45.16 years (95% CI: 33.51-56.82) and those given Methotrexate [13-21] with a mean age 47.62 years (95% CI: 39.02-56.41). The minimum duration of follow up was 52 weeks; the mean duration of follow up was 71.33 (95% CI 42.81-99.86) in the control group and 73.14 (95% CI 49.28-98.00) in the treatment group (Figure 2). 6 patients in the non-immune suppressed group and 16 patients in the Methotrexate group had more than one high risk factor for survival of the graft. The number risk factors operative in patients in each group were as shown in pie diagram (Figure 3) failed previous graft & other corneal scars accounted for 10 eyes (83.33%) in the control group against 15 eyes (71.43%) in the immune suppressed group (Table 1). Notably at the end of the follow up period, Grade 4 clear grafts were 1 and 8 in the two groups respectively (Figure 3), while clear and borderline clear grafts in the two groups were 4 and 13. Patients were not willing for more than 1-2 regrafts and the option of a keratoprosthesis was preferred in repeated failures.

Nonparametric Wilcoxon rank sum test was performed to validate the number of rejection episodes; Donor cornea grade, post-operative complications and Graft size of clear grafts are tabulated in Table 2. The duration of Methotrexate use was 31.95 wks on a average (95% CI 23.21-40.69) (Figure 4). Since the duration of follow up varied, we tried to consider the ratio of duration of Methotrexate use to the follow up duration and its relation to graft status (Table 3, Figure 5). Patients with grade 4 clear grafts had a ratio of 0.658 (95% CI: 0.3690-0.9469) as compared to failed grafts which had a ratio of 0.2396 (95% CI: 0.1224-0.3569). Only one patient had to be discontinued due to anaemia which was detected as a part of our routine monitoring protocol.

Patients on systemic Methotrexate were found to have a statistically significant chance of having a grade 4 clear graft as compared to the control group. P value=0.0409 More specifically it was found that patients with two high risk factors on systemic Methotrexate were found to have a statistically significant chance of having grade 4 clear graft as compared to the control group. (P value=0.0308) and as compared to those who had only one risk factor

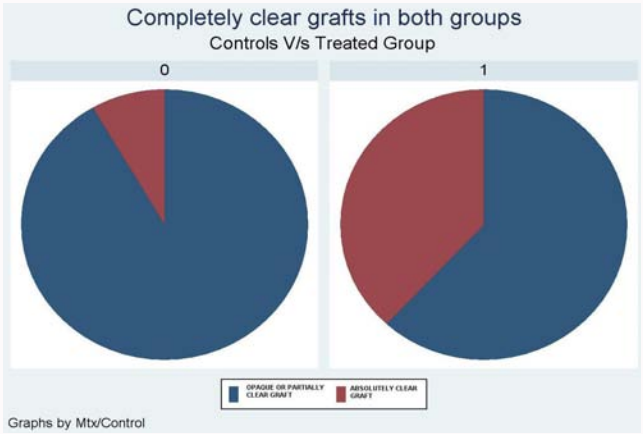

Figure 3: Completely clear grafts in both groups.

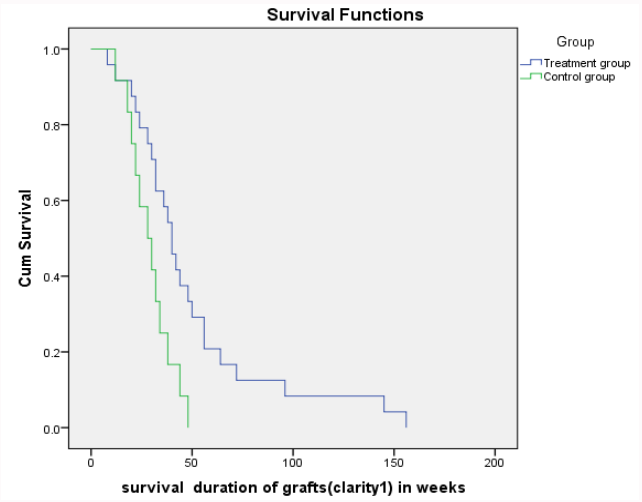

Figure 4a: Survival Graphs.

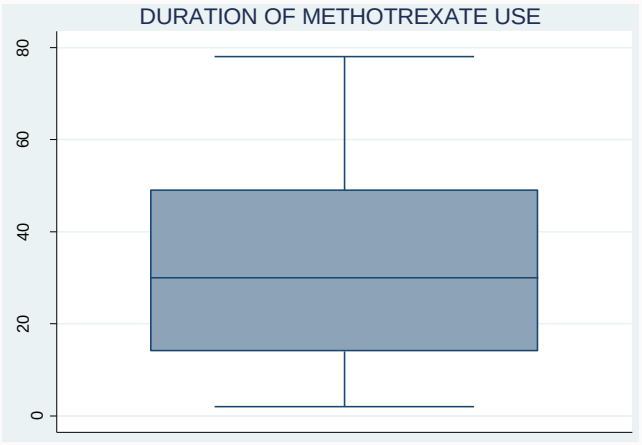

Figure 4b: Duration of Methotrexate use.

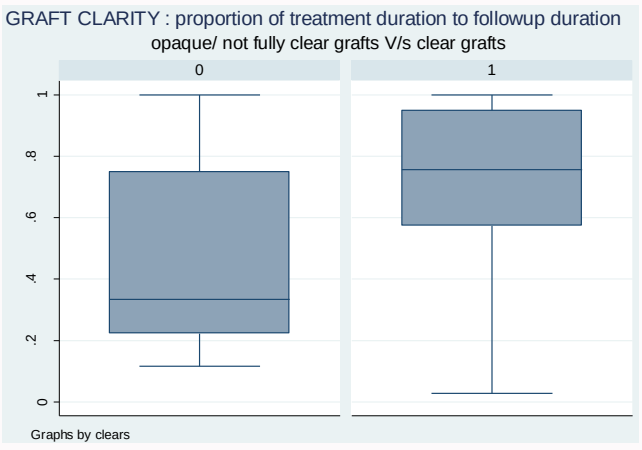

Figure 5: Grafts clarity: Proportion of treatment duration to followup duration opaque/not fully clear grafts V/s clear grafts.

(P value=0.0662).

There was no statistically significant difference in the distribution of eyes with recurrent epithelial defects P=0.2979, secondary glaucoma p=0.1948, HSV etiology=0.8666 and pre op dis-organized anterior chamber P=0.4497 in the two groups being considered.

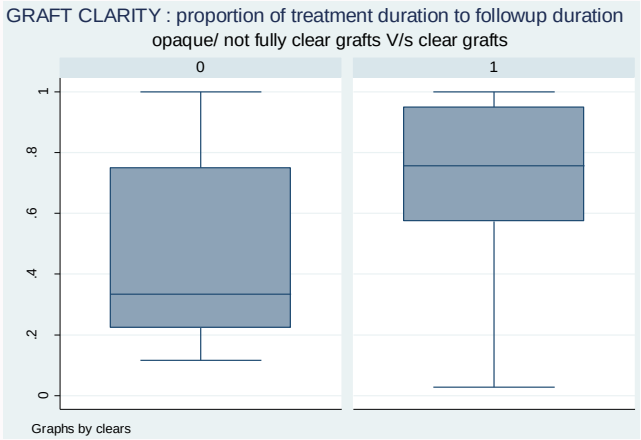

Figure 6: Graft clarity: Proportion of treatment duration to followup duration opaque/not fully clear grafts V/s clear grafts.

There was also no statistical difference in the grade of donor tissue used in the two groups P=0.2774.

Discussion

Corneal transplant is the most successful and therefore perhaps the most commonly performed solid organ transplant [8]. The outcome of penetrating Keratoplasties in first time grafts in 'low risk ' settings is quite favorable, with graft survival rate around 90% at one- two year follow up. However this is the situation for absolutely quiet, non-inflamed eyes with avascular corneas as in keratoconus and other corneal stromal dystrophies. The picture is quite different for 'moderate risk' or 'high risk' cases. There is no universally accepted definition of high risk Keratoplasties but various reports in the literature suggest that recipient corneas can be divided into low, medium and high risk categories depending on the no. of quadrants of vascularization *viz.* a vascular, 1-2 quadrants & 3 or more quadrants [3]. Topical corticosteroids which are the gold standard in the management of post PK patients have shown unsatisfactory results in such cases. The efferent pathway for allograft rejection consists of clonal expansion of graft specific cells in lymphoid tissue. As topical steroids do not reach and even systemic steroids do not interfere sufficiently with proliferation of T cells, it is essential to administer systemic immunosuppressive in order to achieve graft survival [9]. But unlike in other solid organ transplants, the need for systemic immunosuppression is a debatable question. For people requiring organ transplants, life of a patient is at stake, so risk of complication from systemic immunosuppression is a lesser evil whereas in corneal transplants, except for poor vision patients are generally healthy so side effects of these drugs are not easily justifiable. Perhaps this is the reason that the need for systemic immuno suppression is not widely accepted even in high risk corneal grafts [10].

The evidence supporting systemic immunosuppression is limited mostly to use of calcineurin inhibitors cyclosporine, FK506 or antiproliferative agents like azathioprine or mycophenolate moefitil. Most of these studies are retrospective case series, with different criterias used to define high risk grafts (Table 3). Results with cyclosporine have been inconsistent. Hill et al. reported improved long & short term survival in high risk cases on cyclosporine [11], whereas a retrospective case control study by Poon AC et al. found that benefit of cyclosporine over conventional therapy in preventing rejection episodes and subsequent failure was only moderate & not statistically significant [12]. On the other hand a prospective study on systemic cyclosporine in high risk corneal transplants [13] has found no remarkable difference in either rejection or graft clarity in 40 eyes of CsA and control group [13]. A study by H Dua et al. [14] showed that with Tacrolimus (FK506) the median graft survival was significantly better and irreversible graft rejection was very significantly low [14]. However this too has been a retrospective case series. Another study by Reinhard et al. [15] showed 83% immune reaction free graft survival in MMF group compared to 64.5% in control group, after a mean follow up of 34.9 +/- 16.3 months. However in all these cases high risk cases had either or both these factors *viz.* 1) deep vascularization 2) previous multiple failed grafts (Table 3).

In our case series in 75% (n=22) patients there have been multiple risk factors. Failed graft with totally opaque corneas with no view of AC [7], failed grafts with disorganized AC [7], failed grafts with florid neovascularization [8]. 16 of our patients were bilaterally blind, with the grafted eye being the one with some visual potential. So most of our patients fell into the' highest' of the high risk category.

Methotrexate, a Folic acid antagonist has been used extensively in systemic immune suppression for decades. It has also been used in a variety of autoimmune ocular conditions. It is known to have a good safety profile and in the context of our patient population is affordable. Methotrexate has been used in clinical practice since the last 70 years. It was introduced in 1950s as a cytotoxic agent for treatment of malignancies due to its action of inhibition of purines and pyrimidines synthesis. It was reintroduced in clinical rheumatology in 1980s as a DMARD (Disease Modifying Anti Rheumatic Drugs) in Rheumatoid Arthritis. Its proposed mechanisms of action are antiproliferative by inhibiting DNA synthesis, diminution of size & reactivity of T lymphocytes by induction of apoptosis, promotion of adenosine release which mediates suppression of inflammation [16]. In ophthalmology, it has been used in peripheral ulcerative keratitis and necrotizing keratitis in Rheumatoid Arthritis [17], Mooren's ulcer [18], ocular cicatricle pemphigoid [19]. And in different types of uveitis [20] there are case reports of methotrexate being used postoperatively in tectonic corneal grafts done for peripheral corneal melts and in high risk P.K.s. While methotrexate has been used for the management of autoimmune conditions like rheumatoid arthritis systemically and ophthalmic conditions enumerated above, few references exist that cite the use of this molecule in penetrating Keratoplasty [21,22].

Our outcome measure was grade 4 graft clarity. We did not consider borderline graft clarity as a successful outcome measure because this would lead to loss of objectivity of the observation. At the level of inter and intra-observer evaluation to grade graft clarity we noticed wide fluctuations in the reporting of the borderline clear group. Graft clarity being our outcome measure reasons other than immunological rejection that can play a role in graft opacification had to be considered. Ocular surface instability, reactivation of HSV infection, poor donor material, Secondary Glaucoma and disorganized anterior segment preoperatively (resulting in angle damage and risk of deep vascularization) can be important confounders causing graft opacification. Skewed distribution of these variables in the treated and control group can generate erroneous results. Therefore these were identified and analyzed. No statistically significant difference in distribution in the two groups was found, strengthening our hypothesis testing. Vision was not considered an outcome measure since it may be affected by other co morbid conditions and is not

Table 1: Comparison of Methotrexate and Control groups.

	Controls -12	Mtx Group- 21
Age (in years)	45.16 (95% CI:33.51-56.82)	47.62 (95% CI:39.12-56.11)
Duration of follow up(weeks)	71.33 (95% CI:42.81-99.86)	73.14 (95% CI:49.28-98.00)
Presence of multiple risk factors	6	16
Risk factors		
Regraft	3	1
>quadrant vascularisation	1	3
HSV scar with deep vessels	0	1
Anterior Synechiae	1	0
Disorganised anterior segment	1	0
>one risk factor	6	16
Indication for surgery		
Failed previous graft	5	12
Other corneal scars	5	3
Viral corneal scar	1	3
Chem. Burns/STS/OCP	0	1
Ocular surface disease	0	2
>one risk factors	1	0
Graft status		
Clear	1	8
Borderline clear	3	5
failed	8	8
Rejection episodes		
None	3	8
One	7	12
Two or more	2	1
Donor cornea grade		
Optical	12	18
Borderline optical	0	3
Post-operative complications		
Suture related problems	0	1
Rejection	0	1
>one complication	12	19
Graft size 8mm or less	8 (66.6%)	19 (90.48%)
Graft size of clear grafts		
7 mm	0	1
7.5 mm	1	2
8 mm	0	5

Table 2: Duration of Methotrexate use/follow up duration ratio and its relation to graft status.

	Mean	Standard error	95% CI
Mtx duration/FU duration	0.4368	0.0788	0.2725-0.6012
Mtx duration/FU duration (in clear grafts)	0.658	0.1223	0.3690-0.9469
Mtx duration/FU duration (in clear & borderline clear grafts)	0.6931	0.0975	0.4806-0.9056
Mtx duration/FU duration(in failed grafts)	0.2396	0.0495	0.1224-0.3569

directly affected by immune response. In our series 61.9% of grafts in MTX (Methotrexate) group remained clear as against 33% in control group. To our knowledge this is the largest case series of methotrexate use in high risk corneal grafts. Patients with clear grafts had a greater ratio of 0.658 of MTX/FU duration (95% CI: 0.3690-0.9469) as compared to failed grafts which had a ratio of only 0.2396 (95% CI:

Table 3:

STUDY	STUDY DESIGN	DRUG USED	FOLLOW UP PERIOD	% CLEAR GRAFTS	SAMPLE SIZE
Shimazaki et al. [13]	RCT	Cyclosporine	42.7 months	55%	39
Sloper et al. [14]	Non comparative case series	Tacrolimus(FK 506)	24 months	56.50%	23
Birbaum et al. [15]	RCT multi centre	MMF (mycophenolate Mofetil)	34.9 months	83%	98
Poon et al. [12]	Retrospective case control	Cyclosporine	22 months	63.33%	49
Hill JC et al. [11]		Cyclosporine	12 months	76.75%	43
Birbaum et al. [15]	Retrospective	Cyclosporine v/s Mycophenolate	36 months	Cyclosporine:77%	252
				Mycophenolate:87%	

0.1224-0.3569) (Table 3, Figure 6). In other words patients who took Methotrexate for a longer time had better graft survival probably due to restoration of immunological privilege to some degree. It is also interesting to note that though there were more rejection episodes [13] in MTX group than in control [9], most [10] of them were less severe and reversible.

The tendency has been to opt for newer molecules being routinely used on long term basis with other solid organ transplants, notably cadaveric renal transplant. They happen to be the natural choice because of extensive experience in other transplants especially from the point of long term safety and already established monitoring protocols. It is not new to medicine that often a tried and tested molecule often gets buried under the onslaught of newer and 'better" entrants.

The safety of methotrexate has been reported by various studies. It can be further understood from the results of the AMBITION study [23] where it was used in a three times higher dose (20 mg per week) where 673 patients with Rheumatoid arthritis were randomized to mono therapy with Methotrexate or tocilizumab for 24 weeks. The incidence of serious adverse events with tocilizumab was 3.8% versus 2.8% with methotrexate (p=0.50), and of serious infections, 1.4% *vs.* 0.7%, respectively. There was a higher incidence of reversible grade 3 neutropenia (3.1% *vs.* 0.4%) and increased total cholesterol > or=240 mg/dl (13.2% *vs.* 0.4%), and a lower incidence of alanine aminotransferase elevations >3x- <5x upper limit of normal (1.0% *vs.* 2.5%), respectively [23]. Methotrexate was found to be the safer drug. Side effects like bone marrow suppression, liver toxicity (elevated serum aminotransferases) and stomatitis are mainly due to its antifolate action and can easily be prevented or reversed by supplementation of 10 mg folic acid daily. In our series too, except for nausea, vomiting and marginal fall in hemoglobin, there were no major side effects that we came across. Our study had only one patient out of 21 patients with a mean follow up of 73.14 weeks needed to be taken of the drug, due to anaemia which was rapidly corrected on discontinuation alone.

An equally important factor in the success of any treatment is the compliance of the patient. Cost of the drug especially for long term usage is important compliance factor for the patient. Needless to say the large percentages of the patients with corneal opacities with high risk factors come from the developing world and war ravaged nations. The availability of a drug which easy to administer, with a good safety profile and affordability can ensure the necessary compliance. Cost Related Non Adherence (CRN) has been adequately documented even in developed countries especially amongst poorer groups and those with chronic disease which limits their earning capacity and needs lifelong treatment [24,25]. The cost of methotrexate which is, less than one tenth the cost of most other immunosuppressants, also becomes a deciding factor in the settings of poor socioeconomic status of patients in a developing country.

A larger sample size, more than one surgeon a longer follow up period would have strengthened the findings of our study. While the use of historical controls operated in the two years prior to the methotrexate group may be controversial. This was done intentionally to prevent a selection bias of assigning patients less at risk to the control arm which would have affected outcomes, if taken from the period. Also the technique used was standard and there was no learning curve in the surgeon who was already well experienced.

Conclusion

Systemic immune suppression with oral methotrexate shows promise in maintaining graft clarity in patients undergoing Keratoplasty, in high risk situations. Its safety profile and cost make it a useful drug especially in the developing world where most of such patients reside. The slightly higher efficacy of cyclosporine and MMF is largely undermined by its unavailability to such patients who need it most, and where methotrexate can be a useful substitute.

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References

1. Williams KA, Muehlberg SM, Lewis RF, Coster DJ. How successful is corneal transplantation? A report from the Australian Corneal Graft Register. Eye (Lond). 1995;9 (Pt 2):219-27.
2. Hill JC. High Risk Corneal Grafting. Br J Ophthalmol. 2002;86(9):945.
3. Hill JC. Immunosuppression in corneal transplantation. Eye (Lond). 1995;9(Pt 2):247-53.
4. The Collaborative Corneal Transplantation Studies Research Group. Collaborative Corneal Transplantation Studies (CCTS). Effectiveness of histocompatibility matching in high risk corneal transplantation. Arch Ophthalmol. 1992;110:1392-403.
5. Saini JS, Reddy MK, Sharma S, Wagh S. Donor corneal tissue evaluation. Indian J Ophthalmol. 1996;44(1):3-13.
6. BSR/BHPR DMARD guideline group. Quick reference guideline for monitoring of disease modifying anti rheumatic drug (DMARD) therapy. 2009.
7. McDonnell PJ, Enger C, Stark WJ, Stulting RD. Corneal thickness changes after high risk penetrating Keratoplasty, Collaborative Corneal Transplantation Studies Group. Arch Ophthalmol. 1993;111(10):1374-81.

8. Report of organ transplant panel, corneal transplantation. Council on scientific affairs. JAMA. 1988;259(5):719-22.

9. Reis A, Birnbaum F, Reinhard T. Systemic immunosuppresives after penetrating Keratoplasty. Ophthalmologe. 2007;104(5):373-80.

10. Coster DJ, Williams KA. The impact of corneal allograft rejection on long term outcome of corneal transplantation. Am J Ophthalmol. 2005;140(6):1112-22.

11. Hill JC. Systemic cyclosporine in high-risk keratoplasty. Short- versus long-term therapy. Ophthalmology. 1994;101(1):128-33.

12. Poon AC, Forbes JE, Dart JK, Subramaniam S, Bunce C, Madison P, et al. Systemic cyclosporine in high risk penetrating Keratoplasty; a case control study. Br J Ophthalmol. 2001;85(12):1464-9.

13. Shimazaki J, Den S, Omoto M, Satake Y, Shimmura S, Tsubota K. Prospective, Randomized Study of the Efficacy of Systemic Cyclosporine in High-Risk Corneal Transplantation. Am J Ophthalmol. 2011;152(1):33-39.e1.

14. Sloper CM, Powell RJ, Dua HS. Tacrolimus (FK506) in the Management of High-risk Corneal and Limbal Grafts. Ophthalmology. 2001;108(10):1838-44.

15. Birnbaum F, Mayweg S, Reis A, Böhringer D, Seitz B, Engelmann K, et al. Mycophenolatemofetil (MMF)following penetrating high risk keratoplasty:long-term results of a prospective, randomised, multicentre study. Eye (Lond). 2009;23(11):2063-70.

16. Genestier L, Paillot R, Fournel S, Ferraro C, Miossec P, Revillard JP. Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells . J Clin Invest. 1998;102(2):322-8.

17. Messmer EM, Foster CS. Destructive corneal and scleral disease associated with rheumatoid arthritis. Medical and surgical management. Cornea. 1995;14(4):408-17.

18. Ashar JN, Mathur A, Sangwan VS. Immunosuppression for Mooren's ulcer: evaluation of the stepladder approach--topical, oral and intravenous immunosuppressive agents. Br J Ophthalmol. 2013;97(11):1391-4.

19. McCluskey P, Chang JH, Singh R, Wakefield D. Methotrexate therapy for ocular cicatricial pemphigoid. Ophthalmology. 2004;111(4):796-801.

20. Gangaputra S, Newcomb CW, Liesegang TL, Kaçmaz RO, Jabs DA, Levy-Clarke GA, et al. Methotrexate for ocular inflammatory diseases. Ophthalmology. 2009;116(11):2188-98.

21. Bertelmann E, Reinhard T, Pleyer U. Current practice of immune prophylaxis and therapy in perforating keratoplasty. A survey of members of the Cornea Section of the German Ophthalmological Society. Ophthalmologe. 2003;100(12):1031-5.

22. Pleyer U. Immunomodulation in penetrating keratoplasty. Current status and perspectives. Ophthalmologe. 2003;100(12):1036-44.

23. Jones G, Sebba A, Gu J, Lowenstein MB, Calvo A, Gomez-Reino JJ, et al. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatiod arthritis: the AMBITION study. Ann Rheum Dis. 2010;69(1):88-96.

24. Lynas K. Study finds drug compliance undermined by barriers of cost. Ann Neurol. 2013;73(2):180-8.

25. Levine DA, Morgenstern LB, Langa KM, Piette JD, Rogers MA, Karve SJ. Recent trends in cost related medication nonadherence among stroke survivors in the United States. Ann Neurol. 2013;73(2):180-8.