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RESEARCH ARTICLE

COMPARATIVE STUDY OF EFFICACY AND SAFETY OF TOPICAL CYCLOSPORINE 0.1% AND TACROLIMUS OINTMENT 0.1% THERAPY WITH FLUOROMETHOLONE 0.1% FOR VERNAL KERATOCONJUNCTIVITIS

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ABSTRACT

Aim: To evaluate and compare the efficacy and safety of eyedrop cyclosporine (CsA) 0.1%, eye ointment tacrolimus (TCA) 0.1% and eyedrop fluorometholone (FML) 0.1% in patients of VKC. **Methods:** Prospective and Interventional Randomised Comparative Double Blinded Study conducted at Baba Saheb Ambedkar Hospital, New Delhi during August 2017 to December 2018.All 60 patients of 5 to 20 years age with moderate to severe VKC randomly divided into three groups A,B and C of 20 patients each and were treated with cyclosporine 0.1% eyedrops, tacrolimus 0.1% ointment and flurometholone 0.1% eye drops respectively. Patients were followed up after 7,14,30,60 and 90 days. Quantitative variables were compared using ANOVA/Kruskal Wallis Test and paired t test/Wilcoxon. Qualitative variables were correlated using Chi-Square test /Fisher's exact test. **Results:** In CsA group symptoms and signs except except foreign body sensation and papillary hypertrophy were decreased earliest at 7 days and these two started improving by 1 month. TCLand FML group showed improvement in all symptoms and signs except papillary hypertrophy after 7 days of treatment. Later started improving by 14 day with FML while it took 60 days with TCA. No changes in fundus and IOP with CsA and TCA was noted whereas, Significant rise in IOP was seen in three patients withFML but no fundus changes. **Conclusion:** FML has early and maximum effect than others, but considering IOP rise seen with it, TCA and CsA can be safely used as an alternative to topical fluorometholone.

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INTRODUCTION

Vernal keratoconjunctivitis (VKC) is a chronic inflammation of the ocular surface usually bilateral and severe in nature and if not treated properly could result in sight threatening results (Bielory, 2000). VKC usually begins in first decade of life and resolves after puberty but chronic severe cases could end up with permanent visually debilitating lesions. This disease shows male predominance with a ratio of 2-3:1 to female. Various study had demonstrated more prevalence in dry hot climates, specifically at the Mediterranean basin, the Middle East, Central and West Africa, India, and South America (Addis, 2018; De Smedt, 2013; Bonini, 2000; Leonardi *et al*., 2006; Leonardi, 2015). However scarce data is available in India (Katelaris, 2011). VKC shows a typical seasonal variation with onset in spring, exacerbation in summer and a tendency towards remission in the autumn-winter period.

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The predominant pattern encountered is a mixed form of disease (72%) with a significant number of patients having a chronic perennial form (36%).Lesser association with atopy and systemic allergies as compared to patients in temperate zones is seen. However, the perennial forms are also reported with exacerbations in the spring-summer period (Singhal, 2018). VKC is an inflammataory disorder of conjunctiva due to infiltration of various inflammatory cells especially eosionphils. Various studies using confocal microscopy have demonstrated involvement of basal epithelium and anterior stromal layer in association of superficial epithelial layers due to decreased density along with increased number of adjacent inflammatory cells (Leonardi, 2012). The pathogenesis of VKC is the amalgamation of interactions between genetic, environmental and immunological factors. The association of VKC with specific HLA haplotype has been investigated within consistent results (Tesse, 2012; Pucci, 2007). Various signs, symptoms and histological findings in many studies point towards an IgE-mediated inflammation playing the major role in the pathogenesis of VKC. Though 50% of VKC patients show worsening of symptoms with allergen exposure but cytological pattern in tears and tissues support the role of

specific IgE–mast cell activation in the development of the disease (Sacchetti *et al*., 2018). Specific IgE are found in serum and in tears during the active phase of the disease. Leonardi *et al*. detected IgE using ImmunoCAP ISAC microarray in lacrimal samples of 10 VKC patients, six showed specific IgE in tears but only three of them had specific IgE detectable in the serum (Leonardi *et al*., 2015). However, the severity score of the disease was not correlated to the presence of serum IgE which indicates towards non-IgE mediated mechanism. Increased numbers of CD4+ Th2 lymphocytes in the conjunctiva and overexpression of cytokines and co-stimulatory molecules have been documented (Abu El-Asrar, 2000; Leonardi, 2000; Shiraki, 2016; Montan, 2002). Overexpression of both Th2 and Th1-derived cytokines, pro-inflammatory molecules, chemokines and growth factors have been also demonstrated (Leonardi *et al*., 2009; Oray, 2013). A recent study showed positive association (30.8%) of VKC with antinuclear antibodies whereas another one documented more strong correlation (about 50%) with a familiar history of autoimmune disorders, suggesting that VKC could be a bridge disease between systemic autoimmune disorders and atopic condition rather than being an isolated ocular inflammation (Occasi, 2015; Zicari *et al*., 2013).

Commonly presenting symptoms in VKC are intense itching, redness, and watering along with photophobia and foreign body sensation (Saboo, 2013). Clinical signs of VKC include a papillary reaction of the upper tarsal conjunctiva, and throughout the limbus sparing eyelid margin (differentiating it from allergic keratoconjunctivitis) (Pucci, 2003). Other typical signs of VKC include bulbar conjunctival hyperemia, discharge along with corneal involvement. Corneal changes are due to the combination of the mechanical injury caused by the friction of the giant papillae of conjunctiva on the corneal epithelium along with the ongoing inflammatory cascade (Kumagai, 2006; Leonardi *et al*., 2012); result of which leads to shield ulcers and plaques causing permanent impaired visual acuity up to 6% of all patients (Leonardi *et al*., 2012; Cameron, 1997). These chronic leasions can lead to secondary complications as microbial keratitis, amblyopia, and rarely to corneal perforation (Kumar, 2009).

Microbial keratitis is one of the most severe complications predominantly with shield ulcer (9-10%). Most commonly isolated bacteria are Staphylococcus epidermidis and Streptococcus pneumonia, followed by Corynebacterium species, Neisseria meningitides; occasionally fungal infections from Aspergillus were also reported (Sridhar, 2003). Keratoconus is another visually debilitating complication of VKC affecting variable population of about 2-15% of patients (Totan *et al*., 2001; Caputo, 2016). A unique and widely shared grading of its severity and specific diagnostic criteria are not well defined leading to underestimation and inappropriate treatment of the disease (Esposito *et al*., 2016). Treatment modalties include pharmacological and non-pharmacological ones. Mild cases of VKC tend to respond with nonspecific and supportive therapy only but chronic cases need steroid drops as first line of management (Gokhale, 2012). Cyclosporine A helps to control ocular inflammation not only by blocking Th2 lymphocyte proliferation and interleukin2 (IL-2) production but also inhibits histamine release from mast cells and basophils and through a reduction of IL-5 production thereby reducing the effects of eosinophils on the conjunctiva (Leonardi, 2005). Tacrolimus (FK-506), a macrolide antibiotic, acts primarily on T-lymphocytes by inhibiting production of cytokines, particularly IL-2, IL-3, IL-5, tumor necrosis factor-α, and interferon-γ. Tacrolimus (FK-506) also acts like cyclosporine A and inhibits activation of T-cells and IgE-dependent histamine release from mast cells and basophils (Schreiber, 1992). Both drugs act on their target cells via cyclophyllin receptors. Studies have shown safe and successful use of of Tacrolimus 0.1% in patients with VKC (Pacharn *et al*., 2007). Though steroid drop is best modality in our armamentarium but also the most dangerous especially with chronic and unmonitored usage leading to complications like cataract and glaucoma. This calls for to look out an alternative to first tine management. Immunomodulators in form of topical ocular preparations of cyclosporine A and tacrolimus have been investigated for treatment. Keeping that in mind, we compared these two immunomodulators to fluorometholone in terms of efficacy and safety.

PURPOSE OF STUDY

To study effect of topical cyclosporine, tacrolimus and fluorometholone in management of VKC in terms of efficacy and safety. To best of our knowledge there is no documented comprehensive study in Indian subcontinent comparing cyclosporine, tacrolimus and fluorometholone in treatment of moderate to severe VKC. The results of current study would help ophthalmic surgeons in providing alternatives to topical steroids for VKC.

MATERIALS AND METHODS

This Prospective, interventional and randomised comparative double blinded study was conducted in department of ophthalmology, Dr. Baba Saheb Ambedkar Medical College and Hospital, New Delhi after taking clearance from local ethical committee during August 2017 to December 2018. A baseline study was taken into consideration and the minimum required sample size was calculated as 20 in each group (total 60 patients) (Bonini *et al*., 2004). All patients of 5 to 20 years age with moderate to severe VKC were randomly divided into three groups A, B and C and were treated with CsA 0.1% eyedrops TCA 0.1% ointment and FML 0.1% eye drops respectively after taking informed consent from patients and parents. Patients using contact lens or with coexisting ocular disease like uveitis, glaucoma, corneal disease, ocular infection and patients who reported hypersensitivity to all three drugs were excluded from the study. Each subject was randomly assigned by sealed envelope system into groups. Group A received the cyclosporine 0.1% drops in a dosage of one drop every 12 hours in both eyes ; Patients of group B received the tacrolimus 0.1% ointment twice a day, and group C received the fluorometholone 0.1% eye drops four times a day, during the 90 days of the study. All patients were evaluated in the subsequent programmed follow up visits (day 7, 14, 30,60, 90). Diagnostic symptoms of VKC included itching, watering, mucous secretions and photophobia while presenting signs were conjunctival hyperemia, papillary hypertrophy, giant papillae, discharge, and Horner-Trantas dots (accumulation of gelatinous inflammatory infiltrates around the limbus) and corneal lesions.Patients with VKC were divided in three clasess mild, moderate and severe on the basis of severity of signs and symptoms (Sanjiv Kumar Gupta1, 2015) (Table-1 and 2). After obtaining demographic information, clinical history and specific symptoms were assessed. Pre- and post-treatment assessments were performed by same ophthalmologist.

Pre-treatment assessments included corrected distance visual acuity (CDVA) using Snellen chart, IOP, Fundoscopic evaluation after pupil dilatation and slit lamp biomicrosopic examination. On each follow-up visit, visual acuity, biomicroscopy, and IOP were recorded. Funduscopy under pupillary dilation was performed only on days 30 and 90.Adverse reactions were noted and needful was done.

RESULTS

The patients age ranged from 5 -15 years ; in group A, mean age was 10.1± 2.2 years, in group B mean age was 10.75 ± 1.97 and in group C mean age was 9.4± 1.93. In group A,there were 20% females and 80%male while in group B & C, ratio was 25% and 75% each. Inter group comparison showed that groups were statistically comparable with regard to age distribution and gender (p>0.05).

Photophobia: It was moderate in 14 cases while severe in 6 cases of group A. Whereas group B and C had 15 moderate and 5 severe patients.

Conjunctival hyperemia was mainly moderate in each group as 95% in group A while 90% in other two groups.

Conjunctival discharge was also moderate in 18,20 and 17 patients in CsA, FML and TCA group respectively rest were mild cases. Both these signs were comparable in both groups, no significant difference was noted. Only 1 patient had corneal infiltrate in FML group. Papillary hyperplasia showed statistical significant difference. 17 patients were having moderate papillary hyperplasia in group A and B while 11 with moderate and 9 with severe hyperplasia seen in group C.

POST TREATMENT RESULTS:

Itching: Significant Improvement was noted in all groups even after 7 days of treatment which progressed till14,30 and 60

Table 1. Grading of Vernal keratoconjunctivitis according to severity of symptoms)

SYMPTOMS	NORMAL	MILD	MODERATE	SEVERE
Itching	No desire to rub	Occasional desire to rub or stretch the eye	Frequent need to rub or stretch the eye	Constant need to rub or stretch eye
Watering	Normal tear production	Positive sensation of fullness of the conjunctival sac without tears spilling over the lid margin	Intermittent ,infrequent spilling of tears over lid margin	Constant or nearly constant, spilling of tears over the lid margin.
Foreign body sensation	Absent	Mild, similar to fine dust sensation	Moderate, similar to sand sensation, with mild tearing and blinking	Severe ,similar to big foreign body sensation,with constant tearing and blepharospasm.
Photophobia	No difficulty experienced	Mild difficulty with light causing squinting	Moderate difficulty, necessitating dark glasses	Extreme photophobia causing the patient to stay indoors; cannot stand natural light

Table 2. Grading of vernal keratoconjunctivitis according to severity of signs)

SIGNS	NORMAL	MILD	MODERATE	SEVERE
Conjunctival hyperemia	Absent	Mild, in an area less than 25% of total conjunctival surface	Moderate	Severe, involving all conjunctival surface
Conjunctival discharge	Absent	Small amount of translucent or whitish discharge in the lower cul-de-sac	Moderate amount of like yellow or green-yellowish discharge in the lower cul-de-sac and in the marginal tear strip	Severe, with blood traces in the lower cul-de-sac and in the marginal tear strip
Tarsal conjunctival papillary hypertrophy	No evidence of papillary formation	Mild papillary hyperemia	Moderate papillary hypertrophy with edema of the palpebral conjunctiva and hazy view of the deep tarsal vessel	Severe papillary hypertrophy obscuring the visualization of the deep tarsal vessels
Corneal infiltrates	No corneal involvement	Fine superficial epithelial defects involving less than half of cornea	Diffuse Fine superficial epithelial defects involving more than half of cornea	Confluent epithelial defects, mucous plaque formation oroval corneal ulcers

Itching: Graded according to classification and was analyzed in all groups. Group A and C had 10-10 patients in moderate and severe class while group B had 5 moderate and 15 severe patients. Itching was comparable in all groups with (p>0.05) throughout the follow up.

Watering: Group A had 14 and 6 patients with moderate and severe watering respectively; while B and C had 16 moderate and 4 severe patients.

Foreign body sensation: Difference in all groups was statistically significant. Pre-treatment FB sensation was primarily moderate in all groups comprising of 18,16 and 13 patients in group A,B and C respectively. Rest 7 patients were severe in group C.

days of treatment ($p < 0.05$). Maximum improvement seen in group C followed by groupB and A.

Watering: After 7 and 14 days of treatment, significant but variable improvement in watering was seen ($p<0.05$). Maximum improvement was seen in group C followed by group B and A. Inter group variations were not significant. After 60 and 90 days of treatment, statistically significant improvement in watering was seen in all groups ($p < 0.05$).

Foreign body sensation: After 7 days of treatment FB sensation was improved ingroup C ($p =0.0001$) and B ($p=0.506$); with maximum improvement in group C. No improvement was seen in group A.

While after14 days of treatment it kept improving in both group B (p <0.0001) and C (<0.0001) but no improvement was seen in group A (P =1). After 60 and 90 days of treatment FB sensation was significantly improved in all groups (p <0.05). Inter group variations were not significant.

Photophobia: After 7 days of treatment photophobia was significantly improved in all groups, maximum in group C (P <0.0001) and least in group A (P =0.014). Results remained same till course of treatment. Inter group variations were not significant in any of above mentioned symptoms.

Conjunctival hyperemia: After 7 days of treatment, it was significantly improved only in group C (p <0.0001). But after 14 days of treatment, statistically significant improvement was noted in all groups (p<0.05). Improvement in group A was less compared to group B and C. This was found almost similar throughout the study (p<0.0001).

Conjunctival discharge: After 7 days of treatment conjunctival discharge was significantly improved in group C (P<0.0001) and B (p=0.0003) but no significant improvement noted in group A (P=0.235). But it started to improve by day 14 in similar manner. After 30 days effectivity was found as TCL > FML>CsA. While after 60 days it again became FML > TCL>CsA and at day 90, scenario changed into FML =TCL >CsA.

Papillary Hypertrophy: This was significantly improved in group C (p =0.001), minimal improvement in group B (p =0.717) and no improvement in group A(p= 1) by day 7. After 14,30 and 60days, group A also showed minimal improvement but not significant (p> 0.05). After 90 days of treatment papillary hypertrophy was significantly improved in all groups p<0.05, FML=TCL>CsA. Coneal infiltrates were present in single patient from FML group and significant improvement was seen after 60 days of treatment and it resolved after 90 days. Inter group variations were not significant among all groups as far as all signs were concerned. Intraocular pressure was raised in three patients of group C after 30 days of treatment which was statistically significant p<0.05, but no significant change was seen in group A and B.

DISCUSSION

Vernal keratoconjunctivitis (VKC) is a bilateral chronic and severe inflammation of the ocular surface; underestimation and illtreatment could end up in sight threatening lesions [(1)]. VKC is a seasonally exacerbated entity presented in both acute and chronic form showing variation in racial, geographical and gender distribution. VKC usually begins in first decade of life and resolves after puberty but chronic severe cases could end up with permanently impaired visual acuity.VKC is usually associated with male predominance in hot arid environment (Addis, 2008; De Smedt, 2013; Bonini, 2000; Leonardi, 2006; Leonardi, 2015). Chronic Patients with moderate to severe disease usually need topical steroids for longer durations leading tosteroid induced complications as posterior subcapsular cataract, increased IOP, corneal changes, and superimposed infections in long run (Sacchetti, 2010). We analyzed the efficacy and safety of topical cyclosporine and tacrolimus in comparison to fluorometholone in treatment of moderate to severe vernal keratoconjunctivitis (VKC) which included 60 patients attending outpatient department presenting with moderate to severe VKC.

Patients were divided in to 3 groups A, B and C as using cyclosporine, tacrolimus and fluorometholone respectively. One patient in group A, while two patients in group B and C lost to follow up. We observed that significant improvement in symptoms (itching, watering,, FB sensation and photophobia) and signs (conjunctival hyperemia, conjunctival discharge, papillary hypertrophy and corneal infiltrate) in all groups. In group A all symptoms, except FB sensation, were decreased. Improvement was seen earliest at 7th day and maximum effect seen one month after treatment. Foreign body sensation started to improve after two weeks of treatment. Improvement in all signs except papillary hypertrophy was noticed earliest after 7 days of treatment and improvement was progressive till 90 days. Papillary hypertrophy started improving after 30 days of treatment. No changes in fundus and IOP after treatment in cyclosporine group were noted. Though mild redness and burning sensation was noted in group A but it relieved after some time.

We found our results consistent with study conducted by Leopodo *et al*. (2010). In group B, improvement in all symptoms was seen after 7 days of treatment and maximum effect was seen after 14 days to 1 month of treatment. Signs in group B, also showed similar pattern of improvement as symptoms except papillary hypertrophy which showed maximum improvement after 60 days of treatment. There were no changes in fundus and IOP after treatment in group B. Our findings were consistent with study conducted by panadda *et al*. (2012). In group C, significant improvement in all symptoms and signs except papillary hypertrophy was observed earliest by day 7 and peak effect was seen after 14 days of treatment. Papillary hypertrophy started improving after 14 days of study and peak effect was seen after 60 days of study. There were no fundus changes after treatment in group C but significant rise in IOP was noted in three patients. Our results were consistent with study conducted by sanjiv kumar *et al*. (2015). On comparing all three groups, earliest and maximum improvement in all symptoms and signs was seen with FML followed by TCA. Comparable results were found between TCA and FML group in all symptoms and signs except papillary hypertrophy.

While cyclosporine had delayed onset and late peak effect compared to TCL and FML group. Comparing adverse events, mild burning and redness was observed, with both CsA and TCL. With FML, significant rise in IOP was seen in 3 patients while no significant change in IOP noted with CsA and TCL. Limitations of our study were short duration and small sample size hence we need large sample size and longer duration of study to authenticate results obtained in our study. After analyzing and comparing all data, we conclude that all three drugs, cyclosporine, tacrolimus and fluorometholone are effective in relieving symptoms and signs in patients of moderate to severe VKC. Though the duration for maximum effect differed. Earliest effect seen with FML followed by TCA and CsA. TCA was more effective than CsA in relieving signs and symptoms. FML caused rise in IOP but neither CsA nor TCA showed any significant effect on IOP. To conclude fluorometholone has early and maximum effect on patients with moderate to severe VKC as compared with tacrolimus and cyclosporine, but considering IOP rise seen in fluorometholone group, tacrolimus and cyclosporine can be safely used as an alternative to topical flurometholone in patients of moderate to severe VKC.

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