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## **Comparative Evaluation of Bepotastine Besilate Versus Olopatadine And Ketorolac Combination on Upper Tarsal Conjunctival Brush Cytology in Patients of Vernal Keratoconjunctivitis**

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### **Abstract**

Comparative evaluation of bepotastine besilate versus olopatadine and ketorolac combination on upper tarsal conjunctival brush cytology in patients of vernal keratoconjunctivitis. This study was a prospective, open label, randomized, comparative clinical study. 100 patients of vernal keratoconjunctivitis between 6 to 20 years of age of either sex willing to give informed consent were enrolled in the study. In Group 1, 50 patients received Bepotastine besilate (0.15%) eye drops twice daily for 8 weeks whereas in Group 2, 50 patients received Olopatadine (0.2%) and Ketorolac (0.4%) combination eye drops twice daily for 8 weeks. Upper tarsal conjunctival brush cytology for eosinophil count was done in both the drug groups during the baseline and at 8<sup>th</sup> week during the treatment. It was found that after the 2 months of drug therapy, patients in both the groups showed reduction in the eosinophil count. However, there was no statistically significant difference between the two treatment groups at the baseline and at 8<sup>th</sup> week. There was 32% reduction in group A versus 28% reduction in group B in eosinophil count at the end of 8<sup>th</sup> week compared to baseline. Both bepotastine besilate versus olopatadine and Ketorolac combination were found to be effective in reducing the eosinophil count in patients of VKC.

**Keywords:** Bepotastine besilate, Ketorolac, Olopatadine, Upper tarsal conjunctival brush cytology, Vernal keratoconjunctivitis

## **Introduction**

Vernal keratoconjunctivitis (VKC) is an atopic condition of the external ocular surface of the eye. It is a severe allergic disease that affects the conjunctiva and sometimes the cornea of both eyes. It is most common and most severe in hot, dry environments such as the Mediterranean basin, West Africa and the Indian subcontinent.<sup>1</sup> In these areas, upto 3% of eye clinic patients present with VKC and 10% of outpatient appointments are made for signs and symptoms related to VKC.<sup>2</sup> VKC commonly occurs in school-age children. A male preponderance has been observed, especially in patients under 20 years of age, among whom the male is to female ratio is 3:1 whereas the ratio in older than 20 years of age is 1:1.<sup>3</sup>

VKC is characterized by recurrent inflammation of the conjunctiva and cornea causing intense ocular symptoms such as redness, itching, photophobia, and mucous discharge with corneal involvement and potential impairment of visual function.<sup>4</sup> In 1988, Buckley coined the term “morning misery” for VKC which described the active disease state of patients with severe discomfort, blepharospasm and mucous discharge from eyes leaving them incapacitated upon awakening and “frequently resulting in lateness for school”.<sup>5</sup> Because conjunctivitis typically shows recurrence in spring time, it is named as vernal.

On external examination, the lids can be erythematous and thickened. The classic finding of giant papillae of more than 1 mm diameter is located most commonly on the upper tarsal conjunctiva. The tarsal conjunctiva develops a cobblestone appearance and in active disease, can have mucus accumulation between the papillae. In the limbal form, the conjunctiva may show gelatinous limbal papillae associated with epithelial infiltrates called Horner-Trantas dots. These are focal collections of degenerated eosinophils and epithelial cells. The cornea may become involved in VKC, and the corneal changes ranges from mild (punctate epithelial erosions) to severe (macroerosions and ulcers).<sup>6</sup>

Histologically, VKC is characterised by the infiltration of lymphocytes, neutrophils, mast cells, eosinophils and basophils to the conjunctiva. In one of the study by Miyoshi T et al,<sup>7</sup> it was reported that the percentages of eosinophils and neutrophils in tarsal conjunctival brush cytology samples in patients with vernal keratoconjunctivitis were significantly higher in

subjects with severe corneal lesions such as ulcers. Inflammatory cells infiltrating from the tarsal conjunctiva are therefore thought to have an important role in the pathogenesis of corneal lesions. The high presence of eosinophils in tear fluid and in the conjunctival tissues shows that eosinophils play a major role in the development of this disease.<sup>8</sup> Eosinophils, when activated by specific and non-specific stimuli, release granule stored, pharmacologically specific proteins, enzymes, and newly formed mediators. Eosinophil cationic protein (ECP) comprises 30% of the eosinophil granule matrix.<sup>9</sup> Its toxic effect on human corneal epithelial cells has recently been demonstrated in vitro. ECP levels in biological fluids correlate with the severity of some allergic diseases and its presence in biological fluids and tissues is now considered a marker for eosinophil activation.<sup>10</sup>

VKC is not difficult to diagnose by clinical examination of the eye. Horner-Trantas dots and large cobblestone papillae are indicative of this condition. VKC is differentiated from other ocular allergic conditions through a comprehensive clinical history and ophthalmic examination. Conjunctival scrapings or tear cytology can be useful, revealing increased leukocytes in the conjunctiva, particularly eosinophils.<sup>11</sup>

In VKC, first line treatment includes allergen identification and avoidance, avoidance of eye rubbing and contact lens wear during symptomatic periods, treatment of tear film dysfunction, cold compresses, topical dual-acting antihistaminics or mast cell stabilizers, oral non-sedating anti-H<sub>1</sub> antihistaminics and treatment of coexisting allergic rhinitis (AR). Second line treatment consider preservative-free topical therapy, short course of topical steroids and oral steroids and allergen immunotherapy (AIT) given by subcutaneous or sublingual route. Third line treatment includes topical immuno-modulators i.e, calcineurin inhibitors, omalizumab which is an anti-IgE monoclonal antibody is prescribed in severe cases of VKC or AKC, especially in the presence of concurrent asthma or chronic urticaria.<sup>12</sup>

Mast cell stabilizers are the first-line drugs in management of VKC. Olopatadine (0.1%) is the first dual acting anti allergic drug to receive FDA approval as both an antihistaminic and a mast cell stabilizer, potentially reducing the need for multi-drug therapy. Its dual mechanism of action is an advantage and the drug may be used both as a therapeutic and prophylactic agent.<sup>13</sup> It also confers the drug superior in terms of clinical effectiveness, rapid onset and longer duration of action. Its dosing regimen is one drop topical instillation twice daily. It has been shown to be

effective against ocular pruritis up to 8 hours. It is well tolerated and can be used in children 2 years and older. The most frequently reported side effects were dry eye, pruritis, stickiness and taste perversion.<sup>14</sup>

Non-steroidal anti-inflammatory drug (NSAIDs) such as Ketorolac works through the inhibition of cyclo-oxygenase enzyme, which produces prostaglandins. It is used as additive drugs to reduce the conjunctival hyperemia and itching related to prostaglandin D<sub>2</sub> and prostaglandin E<sub>2</sub> production. Ketorolac (0.5%) is approved by US-FDA for both SAC and VKC.<sup>15</sup> Application of topical NSAIDS is limited due to stinging and burning sensation. Despite these facts Ketorolac tromethamine formulation has shown significant effectiveness in the treatment of acute allergic conjunctivitis.<sup>16</sup>

Bepotastine besilate is a newer anti-allergic agent with multiple mechanism of action. It is a dual acting agent as it is highly selective histamine (H<sub>1</sub>) receptor antagonist with potent mast cell-stabilizing effects. The anti-inflammatory actions of bepotastine besilate include inhibition of leukotriene B<sub>4</sub> production and attenuating eosinophil chemotaxis and activation.<sup>17</sup> The conjunctival allergen challenge (CAC) based clinical trials established that bepotastine besilate ophthalmic solution (BBOS) (1.0% or 1.5%) provided a statistically and clinically significant reduction in ocular itching for up to 8 hours post-instillation in clinical trials as well as statistically significant reductions in conjunctival hyperemia associated with allergic conjunctivitis.<sup>18</sup>

Topical Corticosteroids can also be used in more severe variants of ocular allergy. Corticosteroids possess immunosuppressive and anti-proliferative properties but they have some limitations like elevation of intraocular pressure and cataract formation.<sup>19</sup>

## **Material and Methods**

This was a prospective, open label, randomized, comparative clinical study. The present study was conducted by the Department of Pharmacology, Regional Institute of Ophthalmology and Department of Pathology, Pt. B.D. Sharma PGIMS, Rohtak. In present study patients of either sex between 6 to 20 years of age who attended the OPD in Ophthalmology department with vernal keratoconjunctivitis were selected. The study was conducted over a period of 1 year and 100 patients were included. Study was done in accordance with the principles of Good

Clinical Practice (ICH-GCP) and Declaration of Helsinki. An informed consent was obtained from all patients enrolled for the study. The study was approved by Institutional Review Board (IRB).

Each study group minimally had 50 patients and had received either topical eye drops of Bepotastine besilate (0.15%) or Olopatadine (0.2%) with Ketorolac (0.4%) combination treatment for a period of 8 weeks i.e two months. A detailed Ophthalmological history with reference to subjective complaints and clinical signs was obtained from the patients at week 0 and followed up at week 4 and week 8. Secondary parameters were recorded using upper tarsal conjunctival brush cytology to see for the eosinophil count at first visit (week 0) and at week 8.

### **Efficacy Parameters**

#### **Upper tarsal conjunctival Brush cytology<sup>20</sup>**

It is the technique that collects conjunctival epithelial samples from the patient, clinically. Brush cytology can obtain basal cells as well as superficial cells. Material required is a small brush with very soft bristles. After instillation of paracaine (0.04%) eye drops, the upper tarsal conjunctiva was everted and brushed several times and scrapings were spread on the clean glass slide by the same examiner in all patients. The strength of the pressure applied to the conjunctiva by brush should be moderate. The slides were then air dried and mounted with hematoxylin and eosin(H&E) stain. Brush cytology from the upper tarsal conjunctiva for eosinophil counts was done at baseline and at 8<sup>th</sup> week during the treatment. Cells per slide were graded using a four level system:

- ( - ): no cells
- (1+): 1-5/slide
- (2+): 6-10/slide
- (3+): >10/slide

### **Results**

This study was planned to compare the efficacy of two different regimens, one being topical bepotastine besilate (Group A) and another being topical olopatadine with ketorolac combination (Group B) received twice daily for 8 weeks.

The efficacy assessment was done at baseline and subsequently the patients of VKC were followed at 8 weeks for the upper tarsal conjunctival scraping from both the eyes.

### **Efficacy Parameter**

#### **Upper Tarsal Conjunctival Brush Cytology (Table 1)**

Tarsal conjunctival brush cytology of 100 patients with VKC demonstrated that eosinophils were seen less frequently in scrapings of patients with mild allergic conjunctivitis and were found in only 60% (30/50) in group A and 52% (26/50) in group B of the patients with VKC at baseline i.e. at week 0. At week 8, the percentage of patients with eosinophils cells reduced in 32% of patients (16/50) in group A and in group B reduction in eosinophil count was seen in 28% of patients(14/50).

In Group A, as shown in figure (3a,3b), upper tarsal conjunctival brush cytology shows 2+ eosinophil count i.e. 6-10 cells/slide at baseline and at week 8, the count reduced to 1+ i.e. 1-5 cells/slide, respectively. In Group B, as shown in figure (4a,4b), upper tarsal conjunctival brush cytology shows 3+ eosinophil count i.e. >10 cells/slide at baseline and at week 8, the count reduced to 1+ i.e. 1-5 cells/slide, respectively. Additional mast cells, neutrophils and sheets of epithelial cells were found in patients of VKC in both the treatment groups.

**TABLE- 1**

#### **COMPARISON OF EOSINOPHIL COUNT IN GROUP A AND GROUP B (N=50 IN EACH GROUP)**

	<b>Group A n=50</b>		<b>Group B n=50</b>	
<b>Eosinophil count</b>	<b>Number of patients</b>		<b>Number of patients</b>	
	<b>Week 0</b>	<b>Week 8</b>	<b>Week 0</b>	<b>Week 8</b>
0	20	34	24	36
1+	16	10	10	5
2+	10	6	8	5
3+	4	0	8	4
Percentage	60%	33%	52%	28%

- Values are expressed in percentage at week 0 and at week 8 in both the treatment groups
- Grading of eosinophil count : 0 (no cells), 1+ ( 1-5 cells/slide), 2+ ( 6-10 cells/slide), 3+ ( >10 cells/slide)

**GROUP A: UPPER TARSAJ CONJUNCTIVAL BRUSH CYTOLOGY  
AT BASELINE**

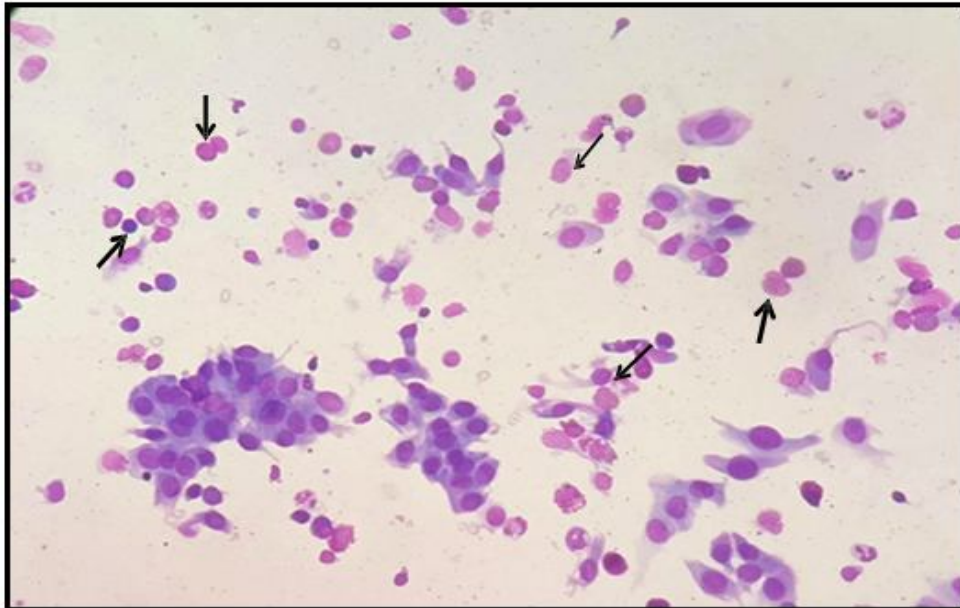


Fig 3a: Showing Eosinophil Count 2+

**GROUP A: UPPER TARSAJ CONJUNCTIVAL BRUSH CYTOLOGY  
AT 8 WEEKS**

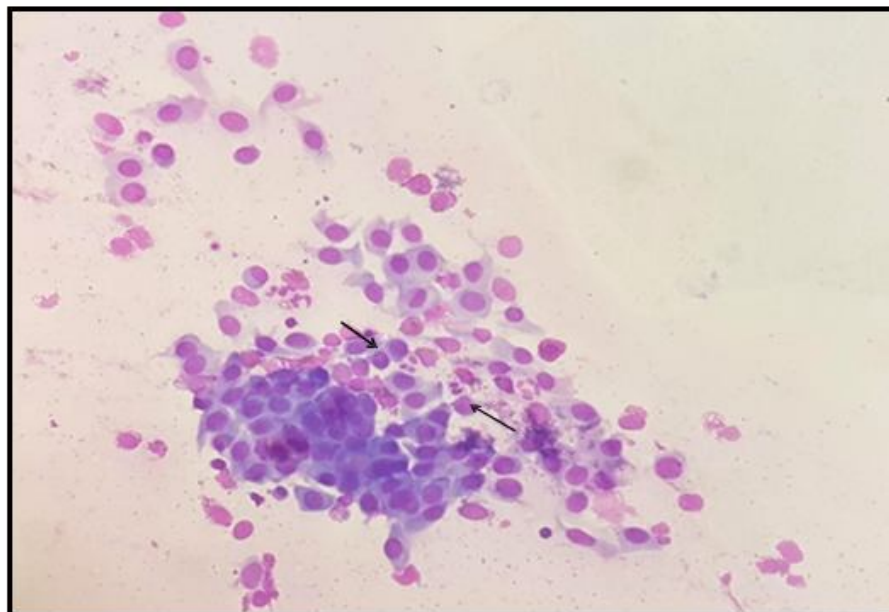


Fig 3b: Showing Eosinophil Count 1+

**GROUP B: UPPER TARSAL CONJUNCTIVAL BRUSH CYTOLOGY  
AT BASELINE**

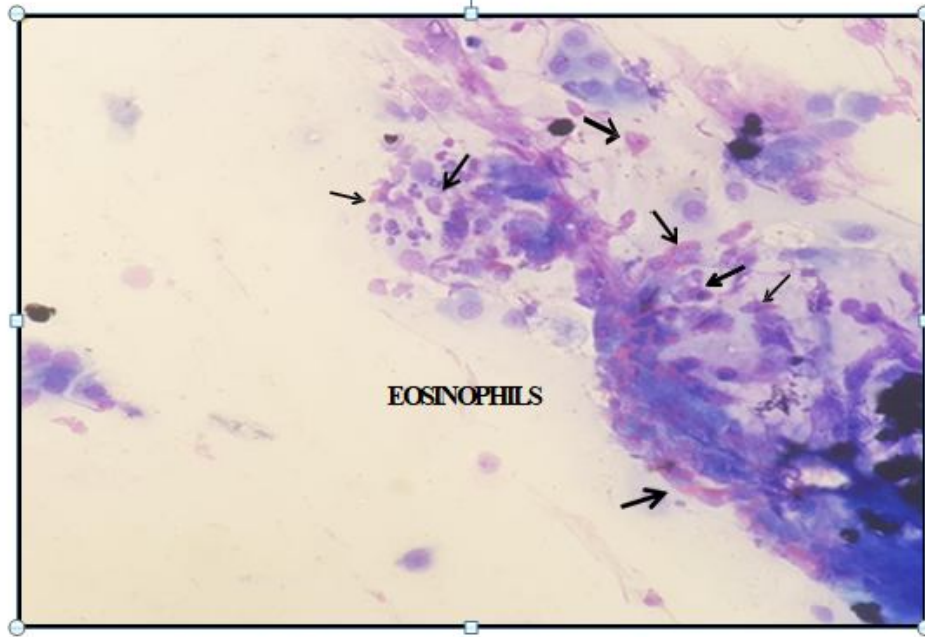


Figure 4a : Showing Eosinophil Count 3+

**GROUP B: UPPER TARSAL CONJUNCTIVAL BRUSH CYTOLOGY  
AT 8 WEEKS**

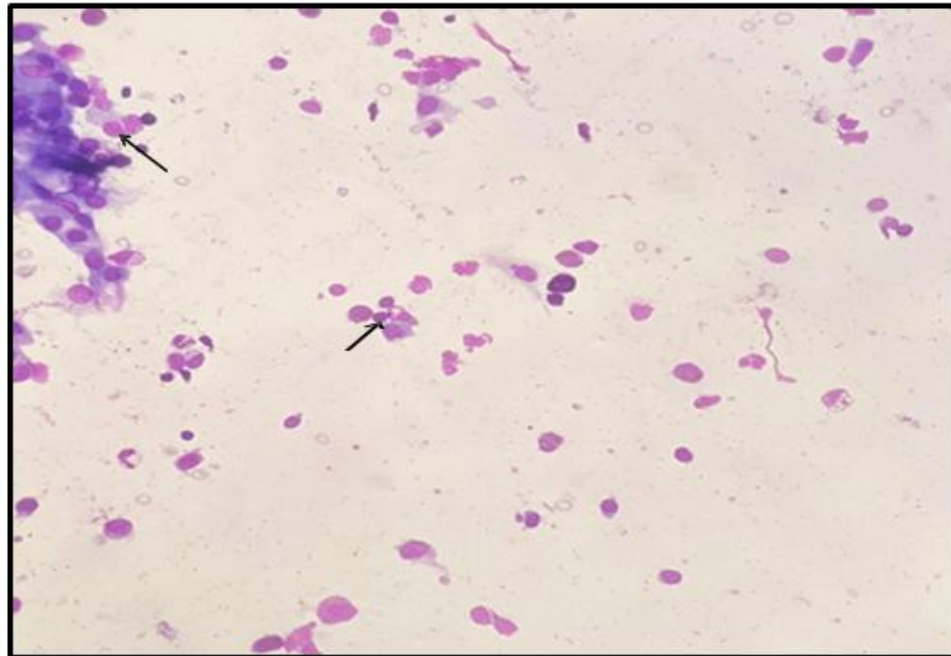


Fig 4b: Showing Eosinophil Count 1+



## Discussion

As per the upper tarsal conjunctival brush cytology, both the groups showed statistically significant reduction in eosinophil count at 8 weeks. The decrease in eosinophil count in group A and B was 32% and 28% respectively when compared to baseline i.e. 60% and 52%, respectively. Both the study groups were comparable regarding the decrease in eosinophil count on upper tarsal conjunctival brush cytology. Both drugs act synergistically to decrease eosinophil count in patients of VKC. The findings of the study matches with the pharmacological profile of monotherapy and combination therapy. The improvement is on the expected lines as new antihistaminics that combine mast cell stabilizing properties and histamine receptor antagonism, such as bepotastine and olopatadine are presently available and show evident benefits in treating all forms of ocular allergy.

The advantage offered by these agents is the rapidity of symptomatic relief in patients of VKC by immediate histamine receptor antagonism, which alleviates conjunctival itching and redness, coupled with the long-term disease-modifying benefit of mast cell stabilization.<sup>21</sup> Also NSAIDS employed in ocular allergy treatment inhibit both cyclo-oxygenase (COX)-1 and COX-2 enzymes.<sup>22</sup> Ketorolac has shown a proven effect on reducing conjunctival itching.<sup>23</sup>

## Conclusion

Both bepotastine besilate and olopatadine with ketorolac combination administered topically in eyes of patients suffering from VKC, were effective in reducing signs and symptoms. On evaluating the secondary efficacy parameter i.e upper tarsal conjunctival brush cytology, it was found that eosinophil count reduced significantly both in Group-A and Group-B at 8 weeks post-treatment. Both the treatments were found to be equally effective with regard to improvement in eosinophil count. Additional mast cells, neutrophils and sheets of epithelial cells were found in cytological specimen in few patients. So, from present study it can be concluded that bepotastine possesses similar efficacy in reducing eosinophil count with olopatadine and ketorolac combination. However, further multicentric studies with larger number of patients are required to reach any definite conclusion regarding superiority of individual drug regimen in patients of VKC.

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