Tear Tryptase in Vernal Keratoconjunctivitis

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Objectives: To determine the tear level of tryptase (a marker of mast cell activation) in vernal keratoconjunctivitis (VKC) before and after treatment. In addition, eosinophil counts in conjunctival scrapings and ocular surface temperature before and after treatment were studied.

Patients and Methods: A total of 20 patients, 7 years or older with VKC, were included in this study. Tear samples for tryptase determination were collected before and 2 weeks after treatment with 4% disodium cromoglycate eyedrops and 0.1% fluorometholone eyedrops. In addition, conjunctival scrapings were obtained for microscopic evaluation, and measurement of the ocular surface temperature was performed before and 2 weeks after treatment. One patient was excluded because the patient did not receive topical treatment. Control tear samples were collected from 20 normal control patients for tryptase determination.

Results: There were 19 patients with VKC (17 males, 2 females). The age range was 7 to 17 years with a mean age of 9 years. The mean number of eosinophils prior to initiation of therapy was 11.37 eosinophils with a range of 1 to 34 per high-power field. Following treatment, the mean number of eosinophils was 3.42 eosinophils per high-power field with a range of 0 to 11 (P<.01). The mean ocular surface temperature for the right eye before treatment was 35.56°C (range, 34.46°C-36.50°C) and after treatment was 33.53°C (range, 31.13°C-35.40°C). For the left eye, the mean ocular surface temperature before treatment was 35.49°C (range, 34.86°C-36.16°C) and after treatment was 33.88°C (range, 32.40°C-35.53°C). The ocular surface temperature was found to decrease significantly following treatment (P<.001). The levels of tryptase in tears of patients with VKC were determined before and after treatment. The mean level was 16.77 ng/mL (range, 5-115 ng/mL). Following treatment with topical 4% disodium cromoglycate and 0.1% fluorometholone eyedrops, the mean level of tryptase decreased to 7.29 ng/mL (range, 5-44.1 ng/mL) (P<.05).

Conclusions: Patients with severe VKC had high levels of tryptase in tears. Following treatment, the level of tryptase in tears decreased significantly.


VERNAL keratoconjunctivitis (VKC) is a form of chronic allergic conjunctivitis that is potentially blinding by the complications of the disease or by the long-term use and abuse of topical corticosteroids. Patients with VKC have an increase in the number of eosinophils, mast cells, and other inflammatory cells in the conjunctiva. The diagnosis of ocular allergy in most cases remains clinical. There are no specific and sensitive diagnostic assays for ocular allergy. Tryptase is a neutral protease that has been shown to selectively concentrate in the granules of human mast cells. It is well known that mast cells play a pivotal role in the pathogenesis of VKC. Determination of granule products of mast cells in tears of patients with allergic conjunctivitis may reflect the degree of activation of the mast cell. The release of tryptase in tears may serve as a clinical marker for mast cell activation and for monitoring of ocular allergy following treatment. Recent studies have shown that mast cells have structural and functional heterogeneity. The distribution and concentration of human tryptase–positive, chymase–negative mast cells and tryptase– and chymase–positive mast cells were examined in the conjunctival biopsy specimens of patients with VKC, giant papillary conjunctivitis, allergic conjunctivitis, and atopic dermatitis. Epithelial mast cells were found in all cases of VKC specimens. Activation of these epithelial mast cells may lead to an increase in tear tryptase levels. Most cells were mast cells and tryptase-positive, chymase-negative...
PATIENTS, MATERIALS, AND METHODS

PATIENTS

Following a minimum washout period of 1 week, 20 patients (18 males, 2 females), 7 years or older with VKC were included in this study. In addition, a control group of 20 age- and sex-matched patients with no history of atopic disorders and no allergic conjunctivitis was included. The protocol was approved by the institution research council, and consent was obtained from each patient’s guardian. One patient was excluded because of discontinuation of topical medications. Patients who were known to be sensitive to topical medications or have secondary infection were excluded before selection. Contact lens wearers were not included in this study. A Schirmer test was performed before and after treatment. Nineteen of 20 patients and 20 control patients had collection of unstimulated tear samples with capillary tubes before and 2 weeks after treatment. Tears and serum samples were collected to determine levels of tryptase among the patients and controls. The ocular surface temperature was measured before and 2 weeks after treatment.

CLINICAL EXAMINATION

The demographic data for each patient were recorded. The patient’s medical history was documented. Medications administered topically or systemically were recorded. A subjective assessment of symptoms was recorded. Physical examinations were conducted by the investigator. The diagnosis of VKC and the grading of signs were made according to the criteria for the diagnosis of VKC previously reported.18 Clinical signs of ocular allergy were assessed and recorded. Patients underwent slitlamp examination. Fluorescein staining of the external eye was assessed. The signs of ocular allergy, including conjunctival hyperemia, edema, punctate keratitis, and Trantas’ dots, were recorded. The temperature of the temporal bulbar conjunctival surface was recorded before and 2 weeks after treatment. The following signs were assessed: hyperemia, papillary hypertrophy, punctate keratitis, and Trantas’ dots. Hyperemia and papillary hypertrophy were graded as follows: 0, none; 1+, mild; 2+, moderate; and 3+, severe. Punctate keratitis was graded as follows: 0, none; 1+, 1 quadrant of punctate keratitis; 2+, 2 quadrants of punctate keratitis; and 3+, 3 or more quadrants of punctate keratitis. Trantas’ dots were graded as follows: 0, no evidence of dots; 1+, 1 to 2 dots; 2+, 3 to 4 dots; and 3+, more than 4 dots. Cases of VKC with a score of 3 or less were graded mild, more than 3 but less than 6 were considered moderate, and more than 6 were considered severe.

LABORATORY DIAGNOSIS AND DETERMINATION OF TRYPTASE IN TEARS

Blood was collected for determination of tryptase before treatment. Two microliters of unstimulated tear fluid collected with capillary tubes were subjected to the determination of tryptase concentration.16-18 Unstimulated tear samples were collected with a capillary tube from 19 patients and 20 controls before and after treatment and stored at −20°C until an assay was performed. The UniCAP 100 instrument (Pharmacia, Uppsala, Sweden) with built-in software was used to process all steps of tryptase assay. The values were expressed in nanograms per milliliter. The detection limit was 0.5 ng/mL. Antitryptase, covalently coupled to Immuno CAP (Pharmacia), reacted with tryptase in the patient specimen. After washing enzyme-labeled antibodies against tryptase, a complex was formed. After incubation, unbound enzyme antitryptase was washed away, and the bound complex was then incubated with a developing agent. After stopping the reaction, the fluorescence in the eluate was measured. The higher the fluorescence value found, the more tryptase was present in the fluid. Following therapy, tryptase assessment in tears was carried out in a random fashion. Tryptase levels in tears were determined 2 weeks following treatment.

MEASUREMENT OF OCULAR SURFACE TEMPERATURE

The ocular surface temperature was measured by a probe (First Temp Genius Model 3000A, Tympanic Thermometer; Sherwood Medical Industries Ltd, West Sussex, England) in each visit. The temperature was measured over the temporal portion of the bulbar conjunctiva without anesthesia. Three measurements were obtained, and the mean was recorded at a room temperature of 21°C. The temperature reading was the mean of 3 readings before treatment and 3 readings after treatment.

CONJUNCTIVAL SCRAPINGS AND TREATMENT

Conjunctival scrapings were obtained before and 2 weeks after treatment. Following instillation of topical anesthetic eyedrops in each eye, the conjunctival scrapings were obtained and placed onto glass slides, fixed with absolute methanol, and stained with Giemsa stain to assess the number of eosinophils per 10 high-power field (HPF). Each patient was given 1 drop of 4% disodium cromoglycate and 1 drop of 0.1% fluorometholone in both eyes 4 times daily for 2 weeks. One patient did not receive the topical medications and was excluded from the study.

A paired t test or nonparametric counterpart analysis was used to compare the pretreatment and posttreatment levels of tryptase, ocular surface temperature, and eosinophil values. P<.05 was considered significant.

RESULTS

A total of 19 patients were included in this study (17 males and 2 females). The age range was 7 to 17 years with a mean age of 9 years. Three patients had mild...
degree of VKC, 7 patients had moderate VKC, and 9 patients had severe VKC. The mean number of eosinophils prior to initiation of therapy was 11.37 with a range of 1 to 34 per HPF. Following treatment, the mean number of eosinophils was 3.42 per HPF with a range of 0 to 11. The mean number of eosinophils in the conjunctival scraping decreased significantly ($P < .01$) after treatment. Table 1 gives the mean ocular surface temperature of each patient before and 2 weeks after initiation of treatment. The mean ocular surface temperature for the right eye before treatment was 35.56°C (range, 34.46°C-36.50°C) and after treatment was 33.53°C (range, 31.13°C-35.40°C). For the left eye, the mean ocular surface temperature before treatment was 35.49°C (range, 34.86°C-36.16°C) and after treatment was 33.88°C (range, 32.40°C-35.53°C). There was a statistically significant decrease ($P < .001$) in the ocular surface temperature after treatment, which correlated with the decrease in conjunctival hyperemia following topical therapy. Eosinophils are known to be major indicators of ocular allergy. They are known to contribute to the ocular surface toxic effects by the secretion of their granules and cytokines such as eosinophilic major basic protein, eosinophilic peroxidase, eosinophilic cationic protein, and neurotoxic proteins. The number of eosinophils was assessed before and after therapy. The number of eosinophils in the conjunctival scrapings specimens showed a decrease in number after treatment. This decrease in the number of eosinophils correlated with the improvement in VKC.

Tryptase is a neutral protease that is selectively concentrated in the secretory granules of human mast cells. Tryptase is released into the ocular surface following the degranulation of the mast cell. In this study, the enzyme-linked immunosorbent assay (ELISA) method was carried out for the detection of tryptase in tears. Eosinophils are known to be major indicators of ocular allergy. They are known to contribute to the ocular surface toxic effects by the secretion of their granules and cytokines such as eosinophilic major basic protein, eosinophilic peroxidase, eosinophilic cationic protein, and neurotoxic proteins. The number of eosinophils was assessed before and after therapy. The number of eosinophils in the conjunctival scrapings specimens showed a decrease in number after treatment. This decrease in the number of eosinophils correlated with the improvement in VKC.

Table 2 gives the tryptase levels in tears before and after treatment in patients with VKC. The mean tryptase level in tears in patients with VKC was 16.77 ng/mL before treatment and 7.29 ng/mL after treatment. The range of tear tryptase levels in severe VKC was 5.2 to 115 ng/mL before treatment and <5 to 44.1 ng/mL after treatment ($P < .05$). The level of tryptase correlated with the severity score of VKC. In the control group, the tear tryptase level was <5 ng/mL in all tear samples. The mean serum level of tryptase in patients with VKC was 6.3 ng/mL (range, 3.9-8.4 ng/mL). The mean serum tryptase level in the control group was 6.8 ng/mL (range, 5.6 ng/mL-13.5 ng/mL). The serum level of tryptase in patients with VKC was within normal limits and similar to the general population despite the elevation in the tears tryptase.

In most cases of ocular allergy, the diagnosis is clinical. This study aimed to find a sensitive and specific test for the diagnosis and determination of treatment efficacy in ocular allergy. The ocular surface temperature was assessed before and after treatment. Conjunctival temperature of patients with VKC was decreased significantly following therapy. In this study, there was a statistically significant decrease in ocular surface temperature after treatment. Ocular surface temperature measurement is a helpful objective indicator for monitoring improvement in ocular allergy. The ocular surface temperature may be a simple, accurate, objective, and sensitive method for the assessment of the prognosis of patients with ocular allergy receiving topical therapy.

**COMMENT**

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described by Schwartz et al, who reported the mean level of serum tryptase in healthy subjects. The level of tear tryptase may be indicative of the exacerbation of ocular allergy. Tryptase in tears seems to be a sensitive assay for the diagnosis of severe forms of VKC and for monitoring the response to treatment.

The release of tryptase into the mucosal surfaces as well as into the circulation serves as a clinical marker of mast cell activation. The current study describes an ELISA method utilizing a newly developed monoclonal antibody for the capture of an epitope in the tryptase in both the serum and tears. In this study, tryptase was found as a sensitive clinical indicator of mast cell activation, and the level of tryptase in tears correlated with the severity of the disease. Increased levels of tryptase were found in patients with severe forms of VKC. The increase in tear tryptase was not associated with an increase in serum tryptase. Tryptase is released from the human mast cell granules on degranulation. Amounts of tryptase less than 1% of those in mast cells have been detected in basophils, but none has been detected in other cell types found in normal human tissues and blood. A tryptase, therefore, is a sensitive indicator of mast cell activity, a cell that plays an important role in the pathogenesis of VKC. The level of tryptase in tears was found to be markedly increased in patients with severe VKC. Following treatment, the level of tryptase decreased significantly in tear fluids of patients with VKC. The determination of mast cell activation in ocular allergy can be substantially improved by measurements of tryptase in tears in patients with VKC. The amounts of tryptase released by mast cells during mild, moderate, and severe allergic conjunctivitis may vary and seems to correlate with the severity of the disease. Tear tryptase levels showed considerable individual variability (Table 2). In 3 cases, the tear levels of tryptase increased following treatment secondary to exacerbation of allergic conjunctivitis and due to treatment failure. All 3 patients, numbers 8, 10, and 17, had worsening of their signs and symptoms following treatment and showed an increase in the tear tryptase levels.

The tear tryptase levels in the serum correlated closely with the drop in the mean arterial pressure of patients who had insect-sting induced anaphylaxis. Similar situations of anaphylaxis have been reported, and the severity of the allergic response did correlate with the tryptase level in the serum. On the other hand, instances of food-induced anaphylaxis show no elevation, suggesting a mechanism that does not involve mast cell activation. Levels of tryptase in bronchoalveolar lavage fluid, skin chamber fluid, and nasal fluid have been used to assess mast cell activation in allergic conditions. Butrus et al described increased levels of tryptase in human tears of patients with allergic conjunctivitis. Although the measurement of tryptase in ocular fluids has obvious clinical utilization, the major deficiency with many of the current methods for measurements is sensitivity. The assay used in this study seems to be a sensitive and specific method for measurements of tryptase to assess mast cell activation in ocular allergy. Repeated freezing and thawing of samples did not seem to affect immunoreactivity. The immunoassay for the determination of tryptase in tears can be performed in less than 1 hour, raising a possibility that tryptase levels could be used to monitor the therapy and disposition of patients in urgent clinical situations. Schwartz et al have reported that serum levels of tryptase in normal subjects range from 1.9 to 4.9 ng/mL. It is likely that the baseline level of tryptase reflects some combination of the total mast cell burden and the rate of mast cell turnover as well as the magnitude of spontaneous subclinical activation of mast cells. It has been shown that minimal variation of tryptase in healthy individuals may be demonstrated. On the other hand, the baseline level of tryptase in healthy subjects seems to be constant over a period of months or years. Certain healthy individuals may have a baseline serum tryptase level of 10 ng/mL or higher. It is not known whether this apparent increase indicates an increase of population of sensitized mast cells or an alternative mechanism for the hyperresponsive state of that individual.

The mean concentration of tryptase in serum was not increased in this patient population, although the tryptase level in tears was increased. This suggests that a localized allergy at a target organ may be too small to increase the serum level of tryptase, and this indicates that these patients have a localized allergy such as VKC without having systemic disease. Rasp et al found that the mean concentration of tryptase in serum was not significantly different from normal controls and subjects with active allergic rhinitis, despite elevated levels of tryptase being present in the nasal fluids. The results obtained by Rasp and associates are similar to what was found in this study, where patients with VKC had elevated levels of tryptase in tears without an increase of levels in serum. This shows that the

### Table 2. Tryptase Levels in Tears Before and After Treatment Among Patients With Vernal Keratoconjunctivitis (VKC)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Initial Grading of VKC</th>
<th>Tryptase, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>1</td>
<td>Moderate</td>
<td>10.0</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>7.97</td>
</tr>
<tr>
<td>4</td>
<td>Moderate</td>
<td>5.7</td>
</tr>
<tr>
<td>5</td>
<td>Severe</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>Severe</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>Severe</td>
<td>14.6</td>
</tr>
<tr>
<td>8</td>
<td>Severe</td>
<td>5.2</td>
</tr>
<tr>
<td>9</td>
<td>Severe</td>
<td>115</td>
</tr>
<tr>
<td>10</td>
<td>Moderate</td>
<td>&lt;5</td>
</tr>
<tr>
<td>11</td>
<td>Mild</td>
<td>&lt;5</td>
</tr>
<tr>
<td>12</td>
<td>Mild</td>
<td>&lt;5</td>
</tr>
<tr>
<td>13</td>
<td>Severe</td>
<td>29</td>
</tr>
<tr>
<td>14</td>
<td>Mild</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>Severe</td>
<td>9.3</td>
</tr>
<tr>
<td>16</td>
<td>Severe</td>
<td>5.2</td>
</tr>
<tr>
<td>17</td>
<td>Moderate</td>
<td>&lt;5</td>
</tr>
<tr>
<td>18</td>
<td>Moderate</td>
<td>6.6</td>
</tr>
<tr>
<td>19</td>
<td>Severe</td>
<td>58.8</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>&lt;5-115</td>
</tr>
<tr>
<td>Mean ± SD, (P&lt;.05)</td>
<td></td>
<td>16.77 ± 27.0</td>
</tr>
</tbody>
</table>
absolute level of tryptase in the circulation does not reflect regional mast cell activation, which is limited to a local tissue site. In certain instances tear samples collected at baseline and at 15 and 60 minutes after the onset of clinical response may show at least a 2-fold elevation in tryptase, providing a greater sensitivity for detecting mast cell activation than examination of a single tryptase sample. In certain clinical situations, however, this is not possible unless if the patient had a level of tryptase determined prior to the onset of the allergic process. It seems that the relative increase in tryptase is a more sensitive indicator of mast cell involvement than the absolute level of tryptase.

In summary, a new immunoassay for tryptase determination in tears has been shown to be sensitive in the detection of tryptase levels in tears. The tryptase level at baseline of patients with VKC may reflect the local responsiveness of the mast cell activation. Following therapy, there was marked decrease in the level of tryptase in tears, which correlated with the improvement in the clinical signs and symptoms. Tryptase levels in tears may serve as a good diagnostic tool for ocular allergy and for monitoring of activity of the disease.

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