Schirmer Test Values and Lysozyme Content of Tears in Acute Dendritic Keratitis

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In acute dendritic keratitis, the lacrimal flow is increased in the affected eye but not in the normal fellow eye. After recovery the tearflow returns to normal. The lysozyme concentration in the affected and the normal fellow eye do not differ statistically significantly nor do they differ significantly from values of an age- and sex-matched control group. There is no correlation between tearflow and lysozyme concentration. These findings support the view of a constant composition of stimulated tear fluid, irrespective of tear volume produced. Invest Ophthalmol Vis Sci 25:55–58, 1984

Not only does controversy exist in the relationship of tear volume and lysozyme concentration, but also in the values of these parameters in eyes with dendritic keratitis and normal fellow eyes.

Eylen et al1 studied the lysozyme tear level in herpetic eye infections. They found a significant decrease of the lysozyme concentration during an acute attack of ocular herpes infections. In the latent period of the disease, the level increased, but it remained lower than in healthy subjects. In their material, both superficial and stromal keratitis, as well as herpetic iritis, were included.

Avisar et al2 studying the lysozyme content of tears in various eye conditions, including a group of patients with acute dendritic keratitis, found an extremely high negative correlation between the amount of tearing and the lysozyme concentration in the tears. They found similar values for the Schirmer test in the inflamed eye and in the fellow eye during active dendritic keratitis; they also found similar concentrations of lysozyme in the inflamed and fellow eye.

Stuchell et al3 found the concentration of lysozyme to be significantly greater in reflex tears than in basal tears.

Mackie and Seal4 and Grabner et al5 did not find any correlation between tear fluid collected in filter paper discs and the lysozyme concentration, which suggests a constant composition of the tear fluid independent of the produced volume.

Therefore, we reinvestigated the relationship of tear volume and lysozyme concentration in acute dendritic keratitis in the inflamed and fellow eye.

Materials and Methods

Informed human consent was obtained prior to undertaking the study. A total of 40 persons, 22 men and 18 women, ranging in age from 8 to 78 years, all with acute dendritic keratitis, were studied. Only patients with positive virus cultures entered the study.

Before treatment, we isolated herpes simplex virus from the cornea and conjunctiva by minimal wiping in all patients. Minimal wiping was done by lightly stroking the area of the dendrite with a cotton swab moistened in GLY medium, a separate swab was used for the conjunctiva. Virus identification was done at the National Institute of Public Health in Bilthoven, the Netherlands. During transport and storage, eye swabs were kept in GLY medium, ie, Hanks' balanced salt solution containing 0.5% gelatin, 0.5% lactalbumin hydrolysate, 0.1% yeast extract, and antibiotics. The specimens were inoculated onto HEp-2 and onto GaBi cells (human diploid fibroblasts) in tubes. Cultures with characteristic cytopathic effect were identified as herpes simplex virus by serum neutralization in GaBi cells. Typing was done in GaBi cells in microplate culture, with the use of rabbit antisera, monospecific for type 1 and type 2, respectively.

Treatment consisted of either trifluorothymidine eye drops or acyclovir eye ointment given five times daily in combination with other supportive measures.

Tear function was estimated in both eyes before treatment (P1), immediately after complete healing6,7 (P2), which was on the average after 9.25 days (8.2–10.3; 95% confidence limits) and 3 months after treatment was initiated (P3).

For the measurement of tearflow, the Schirmer I test8 was carried out in the unanesthetized eye with sterile Whatman No. 41 filter paper strips measuring 5 x 35 mm. The length of wetting of the filter paper strip was measured after 5 minutes, discounting the first 5 mm of the strip that was inserted in the con-
junctival sac, as this amount of wetting represents the average volume of the tearlake. If the filter paper strip was entirely wetted before the time limit, the length of wetting was calculated for 5 minutes by multiplying the ratio of 5 and the number of minutes at which complete wetting was achieved, with 30 mm.

Tear samples to estimate the lysozyme concentration, which was determined by the agar diffusion method, were obtained by inserting a sterile Whatman No. 3 filter paper disc of 6 mm diameter in the cul-de-sac of the unanesthetized eye. The lysozyme concentration was expressed as μg/ml hen eggwhite lysozyme.

The control group consisted of 40 age- and sex-matched persons. For the statistical evaluation of the data, the analysis of variance and correlation were used.

**Results**

In 4 of 44 persons, the virus cultures were negative, these persons were excluded from the study.

In Figure 1, the cumulative frequency distribution of the age of the patients in class intervals of 10 years is shown.

Table 1 summarizes the analysis of the data of the Schirmer I test over the effect of inflammation (diseased versus normal fellow eye), the effect of periods (P₁, P₂, and P₃) and their interaction, ie, those variance components that cannot be accounted for by the variance of the effect of inflammation and the effect of periods.

From this analysis and the breakdown analysis of the data of the affected and normal fellow eye separately, it appears that the Schirmer values differed significantly between the eyes with dendritic keratitis and the normal fellow eyes. The Schirmer values of the affected eye also differed significantly at P₁, P₂, and P₃; however, there was no difference in Schirmer values at the stated periods of the normal fellow eye. From the statistical significance of the I × P interaction, we may conclude that the inflammatory and period effect are not independent in their effect on tearflow, this means that the increased tearflow was the result of the inflammation of the affected eye at P₁ and P₂.

The average values of the Schirmer test for the affected and normal fellow eye are shown in Table 2. If the data of the Schirmer test of the normal fellow eye averaged over P₁, P₂, and P₃ (as there was statistically no difference at these periods) are compared to the Schirmer data of the age- and sex-matched control group, no statistical difference was found. The average Schirmer value for the normal fellow eye, averaged over the periods, is 15.12 mm and for the age- and sex-matched control group, 14.71 mm, with a standard error of the mean of 2.10.

Table 3 summarizes the analysis of the data of the lysozyme concentration in the tear fluid at P₁, P₂, and P₃ for the affected as well as the normal fellow eye and their interactions. There appears to be no statistical difference in lysozyme values between the affected and fellow eye, nor was any difference found for either eye.

**Table 1.** Analysis of main effects: Inflammatory effect, periods, and their interaction. Analysis of variance of all data of the Schirmer test.

<table>
<thead>
<tr>
<th>Nature of effect</th>
<th>Source</th>
<th>Sum of squares</th>
<th>DF*</th>
<th>Mean sum of squares</th>
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<tr>
<td>Main factors</td>
<td>Inflammatory† effect (I)</td>
<td>19458.00</td>
<td>1</td>
<td>19458.00</td>
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<tr>
<td></td>
<td>Periods (P)†</td>
<td>20032.91</td>
<td>2</td>
<td>10016.45</td>
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<tr>
<td>Interactions</td>
<td>I × P†</td>
<td>14835.81</td>
<td>2</td>
<td>7417.90</td>
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<td>between pairs</td>
<td></td>
<td>264784.78</td>
<td>234</td>
<td>1131.56</td>
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<tr>
<td>Total</td>
<td></td>
<td>319111.50</td>
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</table>

* DF = degrees of freedom.† Statistically significant, P < 0.01.
Table 2. The average Schirmer test values in mm wetting of the filter paper strip and lysozyme values in µg/ml hen eggwhite lysozyme of affected and nonaffected eyes at P₁, P₂, and P₃.*

<table>
<thead>
<tr>
<th>Nature of effect</th>
<th>Source</th>
<th>Sum of squares</th>
<th>DF*</th>
<th>Mean sum of squares</th>
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<tr>
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<td>950.25</td>
<td>239</td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of the mean for Schirmer values: 5.32 and for lysozyme values: 369.45.
† A = affected eye; F = normal fellow eye.

Table 3. Analysis of main effects: Inflammatory effect, periods, and their interactions. Analysis of variance of all data of the lysozyme test.

at the stated periods. Also, no interaction was found between the inflammatory and period effect; therefore, these effects are independent in their effect on the lysozyme concentration in tear fluid, this means that the inflammation did not have any effect on the lysozyme concentration of the eyes at the stated periods.

In Table 2, the average lysozyme concentration expressed in µg/ml eggwhite lysozyme of the affected and normal fellow eye are given at the stated periods. The standard error of the mean is 369.45 µg/ml lysozyme.

In Figure 2 the Schirmer values and in Figure 3 the lysozyme concentrations are shown in relation to the stated periods with the 95% confidence limits.

It will come as no surprise that there is no correlation between the data of the Schirmer test and the data of the lysozyme concentration (r = 0.0036). If the data of the lysozyme concentration of both affected and normal fellow eye, averaged over P₁, P₂, and P₃ (as there are no statistical differences between the eyes and at these periods) are compared with those of the age- and sex-matched control group, again no statistical difference is found. Averages are 3,300.66 µg/ml lysozyme for patients and 2,894.19 µg/ml for control persons, respectively.

Discussion

The results of our study are at variance with those of Eylan et al., both with respect to the acute and latent phase. The differences in results in the acute phase may have been due to differences in populations tested. The findings of these authors were based on the study of a group that had a variety of herpetic diseases, such as dendritic keratitis, stromal keratitis, and herpetic iritis. The differences in results comparing the values of the recovered eye with those of a control group in the latent phase may have been due to the selection criteria of the control group. As the age effect on lysozyme concentration in tear fluid is statistically significant, control groups should be at least age matched, which was not specifically stated by Eylan et al.

Differences in population could not have been the origin of the marked discrepancies in the findings of Avisar et al. in their Group 4 and our findings as both were dealing with acute dendritic keratitis. We did not find any difference in lysozyme concentration of the tear fluid between the affected and the normal fellow eyes, nor did we find any difference in lysozyme concentration of the tear fluid between affected eyes and those of an age- and sex-matched normal control group. We also could not find any difference in Schirmer values between normal fellow eyes and the age- and sex-matched control group. Also, no correlation was found between Schirmer and lysozyme values in the eyes, not even in the affected eyes, in spite of a sharp increase in Schirmer values during the acute phase.

Stuchell et al. found the lysozyme concentration increased in stimulated tears. From their data, one would expect some increase in lysozyme concentration in the affected eye as compared to the normal fellow eye.

Jordan and Baum studied the basal secretion of tears and concluded that continuous basal secretion was possible but that its contribution to the total physiological tear volume was very small. Their findings are supported by Scherz and Dohlman, who observed the development of keratoconjunctivitis sicca after partial extirpation of the lacrimal gland, indicating that the remaining secretion and the basal secretion, at least in some patients, is not enough to maintain the corneal integrity.
Fig. 2. Schirmer values in mm wetting of the filter paper strip and 95% confidence limits of the affected eye (solid line) and normal fellow eye (broken line) at \( P_1 \), \( P_2 \), and \( P_3 \).

Fig. 3. Lysozyme concentration expressed in \( \mu g/ml \) hen eggwhite lysozyme and 95% confidence limits of the affected eye (solid line) and normal fellow eye (broken line) at \( P_1 \), \( P_2 \), and \( P_3 \).

Mackie and Seal\(^4\) and Grabner et al\(^5\) did not find any correlation between tear volume in discs and lysozyme concentration. This suggests a constant composition of stimulated tear fluid, irrespective of tear volume produced, which finds additional support in the study of Gillette et al\(^{14}\) on the histologic and immunohistologic comparison of main and accessory lacrimal tissue. They found the lacrimal and accessory tissues histologically identical with identical distribution of secretory products and immunoglobulin containing plasma cells. These authors concluded a common source for unstimulated and stimulated tears. The results of our study seem to support that view.

**Key words:** Schirmer test, lysozyme, tear fluid, dendritic keratitis, herpes simplex virus

**References**