Biological variations of lysozyme concentration in the tear fluids of healthy persons

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SUMMARY Concentration of tear lysozyme was measured in healthy Indian subjects of the same racial group aged 2 to 78 years by a single radial immunodiffusion technique. Variations in its concentration between the two eyes, during the day, over a period of time, in both sexes, and in different age groups were studied. The diurnal and age-related changes in the concentration of tear lysozyme were found to vary to an appreciable extent. The mean lysozyme concentration in tears was lower in the morning and higher in the evening. It tended to be low in children under 16, rose and attained a plateau in the age group 16-45 years and fell in later life, though still remaining higher than in the children.

Lysozyme, discovered by Fleming,¹ is widely distributed in nature. It has been found in measurable quantity in tears, saliva, serum, nasal secretions, urine, neutrophils, and macrophages.² Since alteration in the lysozyme level in human tears has been observed in some ocular diseases,³⁻¹² its measurement can be of considerable diagnostic importance. However, an adequate knowledge of the biological variations is essential for valid comparisons of tear lysozyme levels between healthy and diseased subjects.

The present study was undertaken to find out differences, if any, in the tear lysozyme concentration between the two eyes and also the diurnal variation in the same eye. The concentrations of tear lysozyme over a period of time and in relation to age and sex have also been studied.

Material and methods

The study was carried out on healthy Indian subjects belonging to the same racial group. They were either volunteers or had attended the outpatient department of Guru Nanak Eye Centre, New Delhi, for refraction. They had no ocular or systemic disease. All of them had been in the uniform hospital environment for at least two hours prior to sampling.

In the first part of the study tear samples were

taken separately from each eye of 26 individuals at the same time to study any variation in the lysozyme level between the two eyes. In the second part of the study tear samples were obtained from the right eye in 18 individuals at four-hourly intervals from 6 am to 10 pm to determine diurnal changes while in the hospital environment. Tear samples (right eye, 10 am) from 22 individuals were taken on six different occasions (alternate months) to study variation with time during a period of one year. Finally, for age-sex related changes tear samples (right eye, 10 am) were obtained from 319 individuals (Table 1).

The tear samples were collected by a method described previously.¹³ The samples were stored at -20°C until assayed which was done within three to four days of collection. The level of tear lysozyme

Table 1 Distribution of tear lysozyme level in mg/ml in the two sexes in different age groups

Age in years	Males		Females		t	df	p
	No. of cases	Mean±SD	No. of cases	Mean±SD			
2-15	35	0.81±0.31	25	0.84±0.22	0.41	58	>0.60
16-30	50	1.68±0.66	37	1.65±0.70	0.20	85	>0.80
31-45	50	1.59±0.54	34	1.61±0.54	0.16	82	>0.80
46-60	37	1.30±0.37	26	1.19±0.33	1.19	61	>0.20
61-78	15	1.01±0.40	10	1.00±0.24	0.07	23	>0.90

SI conversion: mg/ml=g/l.

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was measured by a single radial immunodiffusion technique.¹⁴ Monospecific rabbit antihuman lysozyme serum and reference standard were obtained from Kallestad Laboratories, USA. A standard curve was constructed for each plate, and the concentration of tear lysozyme was determined with reference to the standard curve.

Results

The lysozyme level in tears in 26 individuals were: right eye: 1.31 ± 0.49 mg/ml and left eye: 1.27 ± 0.41 mg/ml. There was no statistically significant difference in the mean lysozyme level in tears between the two eyes ($t=0.81$ for 25 df, $p>0.40$). (SI conversion: mg/ml = g/l.)

The tear lysozyme level at different times of the day in 18 persons were: 6 am: 1.35 ± 0.42 mg/ml; 10 am: 1.44 ± 0.39 mg/ml; 2 pm: 1.41 ± 0.36 mg/ml; 6 pm: 1.47 ± 0.36 mg/ml; and 10 pm: 1.52 ± 0.32 mg/ml. Analysis of variance (Table 2) showed that there was a significant variation in the lysozyme level in tears during the course of the day ($F=4.32$ for 4 and 68 df, $p<0.01$). It was found that, when individual comparisons were made, the differences in the lysozyme concentrations in tears that attained statistical significance at the 5% level were those between the concentration at 6 am and that at 6 pm and between the concentration at 6 am and that at 10 pm.

The mean lysozyme levels in tears over a period of one year in 22 individuals were: January: 1.31 ± 0.46 mg/ml; March: 1.23 ± 0.36 mg/ml; May: 1.31 ± 0.33 mg/ml; July: 1.29 ± 0.33 mg/ml; September: 1.30 ± 0.29 mg/ml; and November: 1.21 ± 0.28 mg/ml. Analysis of variance showed that the variations were not statistically significant (Table 3). The mean

'between months' sum of squares was less than the mean 'within months' sum of squares. No formal test of significance was, therefore, found to be necessary.

The tear lysozyme levels in both sexes in the different age groups are given in Table 1. It was noted that differences between males and females were not statistically significant in any of the age groups. The data for the two sexes were therefore pooled for each group before making comparisons by age. The mean lysozyme levels in tears in various age groups are given in Table 1. The increase in the level of lysozyme in tears from the age group 2–15 years to 16–30 years was statistically significant ($p<0.001$). Again, the fall in the level of tear lysozyme from the age group 31–45 years to 46–60 years and from 46–60 years to 61–78 years was statistically significant ($p<0.001$).

Discussion

Valid comparisons of tear lysozyme level between healthy and diseased eyes are not possible without adequate control of biological variations. Several factors may influence the level of lysozyme in tears. A comprehensive study was therefore undertaken, and it showed that diurnal and age-related changes varied to an appreciable extent. There were no significant variations in the levels of tear lysozyme between the two eyes and at different times of the year.

Significant diurnal changes that we found in the level of tear lysozyme are interesting in that one earlier study¹⁵ showed no diurnal variation. No other study on diurnal variation is available for comparison. As regards variations in relation to sex, the present study is in agreement with the work of other investigators who found the level of tear lysozyme to be independent of sex.^{8 10 15 16}

There are several reports on age-related changes in the level of tear lysozyme which are conflicting.^{8 10 17 18} Some investigators have found that there is no effect of age on the tear lysozyme levels.^{8 10 16 17 19} Bonavida and Sapse²⁰ found lower levels of tear lysozyme at the upper and lower extremes of age. Pietsch and Pearlman¹⁵ observed that the level of lysozyme in tears fell continuously after the age of 10 years. Mukai⁹ found that in healthy subjects tear lysozyme reached peak levels in the age group 11 to 20 years and tended to decrease thereafter. Mackie and Seal²¹ also noted a gradual decline of tear lysozyme levels with advancing age. Morsky and Aine²² found a tendency to declining concentrations after 31–45 years of age. McGill *et al.*¹⁸ found that tear lysozyme levels started falling after the age of 40 years at a rate of 1 mg/dl (10 mg/l) per year of age. Our study on a large number of subjects shows that the level of tear lysozyme tends to be low in the

Table 2 Variation in tear lysozyme concentration during the day: analysis of variance

Source of variation	df	Total sum of squares	Mean sum of squares	F
Between periods	4	34.27	8.57	4.32
Between individuals	17	1068.90	62.88	31.76
Error	68	134.93	1.98	
Total	89	1238.10	—	—

Table 3 Variation in concentration of lysozyme in tears over a period of time: analysis of variance

Source of variation	df	Total sum of squares	Mean sum of squares
Between months	5	0.26	0.05
Within months	126	63.88	0.51
Total	131	64.14	—

younger age group (2–15 years: 0.82 mg/ml), rises and attains a plateau in the age group 16–45 years (16–30 years: 1.67 mg/ml; 31–45 years: 1.60 mg/ml), and then falls significantly in later life (46–60 years: 1.26 mg/ml; 61–78 years: 1.01 mg/ml) but still remains higher than that found in younger people. The earlier studies, where no alterations of tear lysozyme level with age were detected, were small and the methods used in them were less sensitive.

Our findings suggest that in studying the lysozyme levels in the tears of diseased people it is necessary to select separate controls matched for age from among healthy people for each disease group, and to collect all the tear samples at a particular time of the day, for valid comparisons to be made.

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