Immunoassay of tear lysozyme in conjunctival diseases

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SUMMARY The tear lysozyme content in 111 normal subjects and in 159 patients with various conjunctival diseases was determined by a single radial immunodiffusion technique. Tear lysozyme level in normal people was 1.33 mg/ml. (SI conversion: mg/ml=g/l.) The mean tear lysozyme levels in patients with chronic irritative conjunctivitis (0.97 mg/ml) and nutritional deficiency with epithelial xerosis (0.76 mg/ml) were significantly lower than in the normal controls. The mean tear lysozyme levels in tears from patients with vernal conjunctivitis (1.20 mg/ml), phlyctenular conjunctivitis (1.10 mg/ml), and acute bacterial conjunctivitis (1.48 mg/ml) were not significantly different from those in the normal controls. Superimposition of acute bacterial conjunctivitis on trachoma did not alter the low tear lysozyme level that existed before in these patients.

Lysozyme (muramidase) is present in high concentration in normal human tears. Its role is uncertain. In view of the bacteriolytic property of lysozyme^{1,2} it has generally been held that the enzyme in tears acts as a protective agent against bacterial infection.

Although there are several studies on the tear lysozyme level in keratoconjunctivitis sicca,³⁻¹⁰ only a few are available on it in other conjunctival diseases.¹¹⁻¹³ The tear lysozyme level in patients with trachoma is low.¹⁴ The purpose of the present study was 2-fold: (1) To investigate whether there is any alteration in the tear lysozyme level in patients with other conjunctival diseases, and (2) To investigate whether superimposition of acute bacterial conjunctivitis in patients with trachoma alters the existing low tear lysozyme level.

Subjects and methods

The study was carried out among 159 patients with various conjunctival diseases and 111 healthy people chosen consecutively from the Outpatient Department of Guru Nanak Eye Centre, New Delhi. The latter group served as control for all the disease groups and comprised people who had no ocular or systemic disease and had attended the hospital only for a routine check-up.

An earlier report by the present authors has already

described the lysozyme level among normal Indian people by the immunoassay method.¹⁴ However, for the purpose of the present investigation it was considered advisable to examine a fresh group of normal people to serve as concurrent control. The diagnosis of conjunctival diseases was based on detailed clinical examination, including slit-lamp biomicroscopy, ophthalmoscopy, and relevant bacteriological and other laboratory investigations. The patients with chronic irritative conjunctivitis were factory workers whose eyes were exposed to noxious fumes for many months. Their chief complaints were watering, burning, stinging, foreign body sensation, and an urge to

Table 1 Pathogenic organisms identified on culture in cases of acute bacterial conjunctivitis

Pathogenic organisms identified	Acute bacterial conjunctivitis* (28 cases)	Trachoma with acute bacterial conjunctivitis† (20 cases)
<i>Staphylococcus aureus</i>	14	13
<i>Pneumococcus</i>	8	5
<i>Pseudomonas pyocyanea</i>	6	3
<i>Streptococcus viridans</i>	5	3
<i>Streptococcus pyogenes</i>	4	4
<i>Proteus vulgaris</i>	3	2
<i>Klebsiella pneumoniae</i>	2	0
<i>Escherichia coli</i>	1	3
Alpha-haemolytic streptococci	2	1

*More than one organism was isolated in 18 patients.

†More than one organism was isolated in 12 patients.

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Table 2 Age and sex distribution of healthy subjects and patients with conjunctival diseases

Sex	Normal	Epithelial xerosis	Vernal conjunctivitis	Phlyctenular conjunctivitis	Acute bacterial conjunctivitis	Chronic conjunctivitis	Trachoma	Trachoma acute bacterial conjunctivitis
Male	55	15	10	13	17	13	16	11
Female	56	10	7	5	11	7	15	9
Total	111	25	17	18	28	20	31	20
Age (yr)								
≤15	20	16	5	14	3	0	2	2
16-30	37	9	12	4	13	9	10	8
31-45	26	0	0	0	11	11	16	8
≥46	28	0	0	0	1	0	3	2
Total	111	25	17	18	28	20	31	20

rub their eyes. Hyperaemia of the conjunctiva was a prominent clinical feature and was particularly marked in the area of the palpebral aperture. On slit-lamp examination there were no follicles, scarring of the conjunctiva, or associated corneal lesions. The pathogens identified on conjunctival smears and cultures in cases of acute bacterial conjunctivitis are given in Table 1. The bacterial infection was mixed in majority of the patients.

A general physical examination was made in every patient, and those who had no ocular or systemic affliction other than the conjunctival disease were chosen. None of the patients received any medication before or at the time of the study.

Stimulated tear samples of 100 to 200 μ l were collected by a method described previously¹⁵ and stored at -20°C until assayed. Lysozyme was quantified by a single radial immunodiffusion technique originally described by Mancini *et al.*¹⁶ after diluting the tear samples 1:25 with phosphate buffered saline (pH 7.4). The technique has been described in detail.¹⁴ Monospecific rabbit antihuman lysozyme serum and the reference standard were obtained from Behring Institute, West Germany. A standard curve was constructed for each plate and the concentration of lysozyme in tear samples was determined with reference to the standard curve.

Results

The age and sex distribution of normal people and the patients are given in Table 2. Tear lysozyme was found in all the samples in measurable quantity. In normal people the overall mean value of tear lysozyme was 1.33 ± 0.69 mg/ml. (SI conversion: mg/ml=g/l.) There was no significant difference ($t=1.07$, DF 109, $p>0.30$) between the levels in males (1.41 ± 0.70 mg/ml) and that in females (1.27 ± 0.67 mg/ml). The tear lysozyme levels in the different age groups are shown in Table 3. It can be seen that the lysozyme level was low in the young, rose with age, and started

falling after the age of 30 years. Since the tear lysozyme level in normal people varied with age, it was necessary for comparison to take separate controls matched for age from among the normal people for each disease group.

The mean levels of tear lysozyme in controls matched for age and in the disease groups are given in Table 4. The mean tear lysozyme was significantly decreased in the cases of nutritional deficiency with epithelial xerosis ($p<0.01$) and in chronic irritative conjunctivitis ($p<0.001$) as compared with normal healthy people of the respective age groups. It was not significantly altered in vernal conjunctivitis, phlyctenular conjunctivitis, and acute bacterial conjunctivitis. The mean tear lysozyme in patients with trachoma with superimposed acute bacterial conjunctivitis was compared with that in patients with trachoma after matching for age, and the difference failed to attain statistical significance ($p>0.05$).

Discussion

The techniques employed for the estimation of tear lysozyme were difficult for routine application and for comparison, and the values obtained varied widely.^{4,5,17,18} Bonavida and Sapse¹⁹ introduced a method based on the bacteriolytic action of lysozyme on the cell wall of *Micrococcus lysodeikticus*. Johansson and Malmquist²⁰ observed that the bacteriolytic

Table 3 Distribution of tear lysozyme level in the various age groups in normal subjects

Age (years)	No. of subjects	Mean $\pm$ SD (mg/ml)	Coefficient of variation (%)
≤15	20	0.83 ± 0.34	41.0
16-30	37	1.64 ± 0.73	44.5
31-45	26	1.45 ± 0.62	42.8
≥46	28	1.24 ± 0.63	50.8

SI conversion: mg/ml=g/l.

Table 4 Tear lysozyme levels in controls matched for age and those in patients with conjunctival diseases

Disease groups	Number of patients and normal controls	Lysozyme in mg/ml					
		Disease groups		Normal controls matched for age		t	p
		Mean $\pm$ SD (SE)	CV%	Mean $\pm$ SD (SE)	CV%		
Epithelial xerosis	25	0.76 $\pm$ 0.44 (0.09)	57.9	1.20 $\pm$ 0.64 (0.13)	53.3	2.87	< 0.01
Vernal conjunctivitis	17	1.20 $\pm$ 0.60 (0.15)	50.0	1.18 $\pm$ 0.66 (0.16)	55.9	0.09	> 0.90
Phlyctenular conjunctivitis	18	1.10 $\pm$ 0.69 (0.16)	62.7	0.98 $\pm$ 0.51 (0.12)	52.0	0.61	> 0.50
Acute bacterial conjunctivitis	28	1.48 $\pm$ 0.66 (0.12)	44.6	1.47 $\pm$ 0.59 (0.11)	40.1	0.09	> 0.90
Chronic irritative conjunctivitis	20	0.97 $\pm$ 0.46 (0.10)	47.4	1.81 $\pm$ 0.81 (0.18)	44.8	4.03	< 0.001
Trachoma	31	0.89 $\pm$ 0.36 (0.07)	40.4	1.52 $\pm$ 0.75 (0.13)	49.3	4.19	< 0.01
Trachoma patients matched for age as control							
Trachoma with acute bacterial conjunctivitis	20	1.06 $\pm$ 0.47 (0.10)	44.3	0.83 $\pm$ 0.38 (0.09)	45.8	1.70	> 0.05

CV = coefficient of variation.

SI conversion: mg/ml = g/l.

lysozyme determination is not a specific enzyme assay and is probably influenced to some extent by factors other than the lysozyme activity. To avoid such influences tear lysozyme levels were measured by a single radial immunodiffusion method.

Anderson¹³ studied cases of epithelial xerosis and found the tear lysozyme to be much lower. Present findings are in agreement with his observations. Thompson and Gallardo¹² and Harada and associates²¹ have shown that the chief sources of lysozyme in tears is the lacrimal gland rather than the mucous gland of the conjunctiva. It is therefore assumed that the cases of nutritional deficiency with epithelial xerosis have a decreased production of the enzyme by the lacrimal gland.

Ridley¹ reported a low level of lysozyme in tears in phlyctenular conjunctivitis and considered it to be due to associated excessive tearing. However, recent studies have shown that there is no correlation between the titre of lysozyme in the tear fluid and the rate of tear flow.^{8,9,11,22} We did not find the tear lysozyme level in this disease to be significantly different from that of healthy subjects.

Erickson and associates¹⁸ reported the tear lysozyme level to be low in eyes exposed to industrial fumes. The present findings are similar. This low level may be due to destruction of lysozyme in the conjunctival sac or to binding of the lysozyme locally to the tissue by noxious agents.¹¹

Reports on the tear lysozyme level in patients with acute bacterial conjunctivitis are contradictory.^{4,12,23,24} Some authors have reported a low level of tear lysozyme in these patients and attributed this to excessive tearing associated with the disease.^{4,23} It has already been pointed out that excessive tearing does

not alter the lysozyme level in tears.^{8,9,11,22} Janke and associates²⁴ on the other hand observed that the lysozyme level was probably slightly higher in acute bacterial conjunctivitis. Gordon and associates²⁵ and McClelland and van Furth²⁶ have demonstrated that the neutrophils contain lysozyme which is liberated from them during degeneration and cell death. Therefore the lysozyme content of tears from patients with acute bacterial conjunctivitis may be expected to be high. However, no significant alteration was found in the tear lysozyme level in such patients as compared with controls. This indicates that the neutrophils present in tears of such patients are not numerous enough to contribute significantly to the lysozyme content of tears.

The tear lysozyme levels in patients with trachoma have been reported to be low.¹⁴ This low lysozyme level in patients with trachoma probably makes them more vulnerable to secondary bacterial infections, which are frequently associated with trachoma. Ridley²⁷ had stated that while the normal lysozyme content of tears might be effective against some pathogenic bacteria, the lysozyme level needed to be decreased only very slightly to be totally ineffective against all pathogens. A group of patients with trachoma were examined as concurrent controls for the group of patients with trachoma superimposed with acute bacterial conjunctivitis. There was no significant difference in the tear lysozyme levels between the 2 groups. This again suggests that acute bacterial conjunctivitis does not alter the lysozyme level in tears significantly.

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