

# Immunoassay of tear lysozyme in acute adenovirus conjunctivitis

A K GUPTA, G S SARIN, P A LAMBA, AND P D'SOUZA

*From the Guru Nanak Eye Centre and Maulana Azad Medical College, New Delhi, India*

**SUMMARY** The tear lysozyme levels were measured by immunoassay in 92 healthy subjects and 98 patients with acute adenovirus conjunctivitis. They were found to be significantly decreased during the acute phase of the disease. The extent of this decline in the tear lysozyme level was correlated with increased severity of disease. There was no significant difference in the tear lysozyme level in viral isolation-positive and isolation-negative patients. The tear lysozyme level showed return to normal levels with clinical improvement.

Lysozyme is an easily measurable component of human tears and is of diagnostic value in certain ocular diseases.<sup>1</sup> Tears deficient in lysozyme have been described in Sjögren's syndrome,<sup>2,3</sup> keratoconjunctivitis sicca,<sup>4,7</sup> smog irritated eyes,<sup>8</sup> herpes simplex viral eye infection,<sup>9</sup> bacterial conjunctivitis,<sup>10,11</sup> trachoma,<sup>12</sup> lepromatous leprosy,<sup>13</sup> corneal ulcer,<sup>14,15</sup> and protein calorie malnutrition.<sup>16</sup>

The tear immunoglobulin,<sup>17</sup> serum immunoglobulin,<sup>17</sup> and complement component<sup>18</sup> levels have been estimated by us in patients with acute adenovirus conjunctivitis. However, tear lysozyme levels have not been studied. Here we report our observations on tear lysozyme levels in patients suffering from adenovirus conjunctivitis in the acute and remission stages. The findings on adenovirus culture and serological titres have been described elsewhere.<sup>19</sup>

## Material and methods

The study was carried out among 92 healthy subjects and 98 patients with acute adenovirus conjunctivitis attending the outpatient department of Guru Nanak Eye Centre, New Delhi. The control group comprised healthy subjects who had no evidence of ocular or systemic disease and who attended the Eye Centre during the same period mainly for the purpose of refraction. In the control group were 48 males and 44 females with a mean age of 34.5 (SD 16.7) years (range 8 to 63 years) and the diseased group comprised 80 males and 18 females with mean age of 28.3 (SD 12.9) years (range 5 to 63 years). The

Correspondence to Dr A K Gupta, VI/6, Maulana Azad Medical College Campus, Kotla Road, New Delhi-110 002, India.

diagnosis of acute adenovirus conjunctivitis was established by detailed clinical examination including slit-lamp biomicroscopy, bacterial culture, viral culture, and specific serological studies. The patients were subgrouped as mild, moderate, and severe according to the intensity of chemosis and congestion. All patients from whom pathogenic bacteria were isolated from conjunctival swabs were subsequently excluded from the present study. Adenovirus types 2, 7, and 8 could be cultured only from 30 out of 98 patients, and the findings are reported elsewhere.<sup>19</sup>

Tear samples were also collected for lysozyme level analysis from 56 patients with adenovirus conjunctivitis during the stage of remission when there was no clinical evidence of the disease.

Stimulated tear samples (100 to 200  $\mu$ l) were collected by the method described previously<sup>20</sup> and stored at  $-20^{\circ}\text{C}$  until assayed. The lysozyme level in tear samples was quantified by a single radial immunodiffusion technique<sup>21</sup> as modified and described previously.<sup>22</sup> Monospecific rabbit antihuman lysozyme serum was obtained commercially from the Behring Institute, West Germany. The reference standard was obtained from Kallestad, USA. The level of lysozyme in tears was calculated from the calibration curve constructed by incorporating three known concentrations of reference standard for every set of lysozyme determinations. The results were statistically analysed by Student's *t* test.

## Results

The tear lysozyme levels in patients with acute

Table 1 Tear lysozyme levels in mg/ml in healthy subjects, acute adenovirus conjunctivitis, and different stages of conjunctivitis

	No. of subjects	Mean $\pm$ SD	Range
Healthy subjects	92	1.45 $\pm$ 0.60	0.32–2.85
Conjunctivitis	98	1.15 $\pm$ 0.50	0.30–2.44
Mild	28	1.36 $\pm$ 0.48	0.32–2.20
Moderate	52	1.13 $\pm$ 0.47	0.32–2.44
Severe	18	0.90 $\pm$ 0.50	0.30–1.92

SI conversion: mg/ml=g/l.

Table 2 Tear lysozyme levels in mg/ml in the acute phase in viral isolation-positive and viral isolation-negative patients with acute adenovirus conjunctivitis

	No. of subjects	Mean $\pm$ SD	Range
Isolation-positive	30	1.07 $\pm$ 0.49	0.32–1.98
Isolation-negative	68	1.19 $\pm$ 0.50	0.30–2.44

SI conversion: mg/ml=g/l.

adenovirus conjunctivitis and healthy controls is given in Table 1. It was 1.49  $\pm$  0.69 mg/ml in males and 1.41  $\pm$  0.50 mg/ml in females and the difference in the two sexes was not significant statistically ( $p > 0.80$ ). There was a significant decrease when the tear lysozyme level in the acute stage was compared with the level in healthy subjects ( $p < 0.001$ ). (SI conversion: mg/ml=g/l.)

Table 1 also shows tear lysozyme levels in mild (1.36 mg/ml), moderate (1.13 mg/ml), and severe (0.90 mg/ml) types of acute adenovirus conjunctivitis. The tear lysozyme level showed a significant decrease in moderate ( $p < 0.001$ ) and severe ( $p < 0.001$ ) types of disease, while its level in the mild type ( $p > 0.50$ ) remained unaltered in comparison with the levels in healthy subjects (1.45 mg/ml).

The tear lysozyme levels in patients who were viral isolation-positive and viral isolation-negative is given in Table 2. A statistically significant difference was found when the level in healthy subjects (1.45 mg/ml) was compared with the level in viral isolation-positive patients ( $p < 0.01$ ) and viral isolation-negative patients, ( $p < 0.01$ ). However, there was no statistically significant difference ( $p > 0.3$ ) between the tear lysozyme level in viral isolation-positive patients (1.07 mg/ml) and viral isolation-negative patients (1.19 mg/ml).

The tear lysozyme levels were determined in 56 patients during the acute and remission stages. During the acute phase the level was 1.11  $\pm$  0.50 mg/ml (range 0.30 to 2.20 mg/ml), and the level rose to 1.32  $\pm$  0.44 mg/ml (range 0.37 to 2.20 mg/ml)

during remission. There was a statistically significant difference ( $p < 0.02$ ) in the levels in the acute and remission stages. There was no significant difference ( $p > 0.20$ ) between the tear lysozyme level in the remission stage and the level in healthy subjects.

## Discussion

The tear lysozyme levels have been measured on the premise that changes in tear protein levels may reflect significant events in acute adenovirus conjunctivitis. Several studies have been reported on the tear lysozyme levels in healthy subjects.<sup>2,10,22</sup> Low levels have been reported in tears from patients with trachoma<sup>12</sup> and lepromatous leprosy.<sup>13</sup> However, tear lysozyme levels have been shown to be unaltered in acute bacterial conjunctivitis,<sup>11</sup> vernal conjunctivitis,<sup>11</sup> phlyctenular keratoconjunctivitis,<sup>11</sup> chronic irritative conjunctivitis,<sup>11</sup> and nutritional xerosis.<sup>11</sup> Tear lysozyme levels have been reported to be significantly low in patients with herpes simplex virus eye infections.<sup>9</sup>

It has been suggested that a low tear lysozyme level in patients with acute bacterial conjunctivitis is due to excessive tearing.<sup>10</sup> The low level of tear lysozyme in patients with acute adenovirus conjunctivitis which we observed in this study may be partly explained by the excessive tearing frequently observed in these patients.

There is a correlation between increasing severity of the disease and the reduction in tear lysozyme level, and there is a return to normal lysozyme level during the remission stage. It is therefore likely that the low level of tear lysozyme in patients suffering from adenovirus conjunctivitis observed in the present study was due to infection and not inherent or pre-existent.

## References

- Copeland JR, Lamberts DW, Holly FJ. Investigation of the accuracy of tear lysozyme determination by the quantiplate method. *Invest Ophthalmol Vis Sci* 1982; **22**: 103–10.
- Avisar R, Menache R, Shaked P, Rubinstein J, Machtley I, Savir H. Lysozyme content of tears in patients with Sjögren's syndrome and rheumatoid arthritis. *Am J Ophthalmol* 1979; **87**: 148–51.
- Erickson O. The absence of lysozyme in Sjögren's syndrome. *Stanford Med Bull* 1955; **13**: 292–7.
- Mackie IA, Seal DV. Quantitative tear lysozyme assay in units of activity per microlitre. *Br J Ophthalmol* 1976; **60**: 70–4.
- McEwen W, Kimura SJ. Filter paper electrophoresis of tears. I. Lysozyme and its correlation with keratoconjunctivitis sicca. *Am J Ophthalmol* 1955; **39**: 200–5.
- Velos P, Cherry PMH, Miller D. An improved method for measuring human tear lysozyme concentration. *Am J Ophthalmol* 1985; **103**: 31–3.
- van Bijsterveld OP. Diagnostic tests in the sicca syndrome. *Arch Ophthalmol* 1969; **82**: 10–4.

- 8 Sapse AT, Bonavida B, Stone W Jr, Sercarz EE. Human tear lysozyme. III. Preliminary study of lysozyme levels in subjects with smog eye irritation. *Am J Ophthalmol* 1968; **66**: 76–9.
- 9 Ronen D, Romano A, Smctana O. Lysozyme tear levels in patients with herpes simplex virus eye infections. *Invest Ophthalmol Vis Sci* 1977; **16**: 850–3.
- 10 Regan E. The lysozyme content of tears. *Am J Ophthalmol* 1950; **33**: 600–5.
- 11 Sen DK, Sarin GS. Immunoassay of tear lysozyme in conjunctival diseases. *Br J Ophthalmol* 1982; **66**: 732–5.
- 12 Sen DK, Sarin GS. Tear lysozyme in patients with trachoma. *Indian J Ophthalmol* 1982; **30**: 139–41.
- 13 Sen DK, Sarin GS. Tear lysozyme in lepromatous leprosy. *Int J Lepr* 1982; **50**: 322–4.
- 14 Avisar R, Menache R, Shaked P, Rubinstein J, Machtley I. Lysozyme content of tears in some external eye infections. *Am J Ophthalmol* 1981; **92**: 555–8.
- 15 Gupta AK, Sarin GS, Mathur S. Tear lysozyme concentration in corneal ulcer. *Afro-Asian J Ophthalmol* 1982; **1**: 29–31.
- 16 Watson RR, Reyes MA, McMurray DN. Influence of malnutrition on the concentration of IgA, lysozyme, amylase and aminopeptidase in childrens' tears. *Proc Soc Exp Biol Med* 1978; **157**: 215–9.
- 17 Gupta AK, Sarin GS. Serum and tear immunoglobulin levels in acute adenovirus conjunctivitis. *Br J Ophthalmol* 1983; **67**: 195–8.
- 18 Gupta AK, Sarin GS. Serum complement component depression during acute adenovirus conjunctivitis. *Br J Ophthalmol* 1984; **68**: 350–2.
- 19 Gupta AK, Prasad AK. Epidemic acute conjunctivitis in Delhi (1981): a virological study. *Afro-Asian J Ophthalmol* 1982; **1**: 62–5.
- 20 Sen DK, Sarin GS, Mani K, Saha K. Immunoglobulins in tears of normal Indian people. *Br J Ophthalmol* 1976; **60**: 302–4.
- 21 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion technique. *Int J Immunochem* 1965; **2**: 235–54.
- 22 Sen DK, Sarin GS. Immunoassay of human tear lysozyme. *Am J Ophthalmol* 1980; **90**: 715–8.

Accepted for publication 18 October 1985.