α_1 -Antitrypsin and serum albumin in tear fluids in acute adenovirus conjunctivitis

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SUMMARY The α_1 -antitrypsin and serum albumin levels in tear fluids were measured by electroimmunodiffusion in 76 healthy subjects and 63 patients with acute adenovirus conjunctivitis. They were found to be significantly increased during the acute phase of the disease. There was a correlation between increased severity of disease and increased levels of both the proteins. There was no significant difference in the tear α_1 -antitrypsin and serum albumin levels in viral isolation-positive and isolation-negative patients. The raised levels of the two proteins in tears may reflect conjunctival inflammation rather than infection, since both isolation-positive and isolation-negative patients had the raised levels. The levels of the two proteins returned to normal with clinical improvement.

α_1 -antitrypsin controls the activity of diverse proteolytic enzymes.¹ It is found in tears, saliva, perilymph, bile, synovial fluid, and amniotic fluid.²⁻⁵ It plays a significant part in regulating the immune response by inhibiting the transformation and migration of lymphocytes.⁶ Its role in corneal ulcer and other corneal diseases has been studied.⁷⁻⁹ However, only a few limited studies are available on its level in patients with conjunctival disease.^{7-9, 10}

The levels of tear immunoglobulin,¹¹ serum immunoglobulin,¹¹ complement component,¹² and tear lysozyme¹³ have been reported by us in patients with acute adenovirus conjunctivitis. Here we report our observations on tear α_1 -antitrypsin level in this disease. Serum albumin levels in tear fluids have been measured because they indicate leakage of serum proteins into tears. The findings on adenovirus culture have been described elsewhere.¹⁴

Material and methods

The present study was carried out among 76 healthy subjects and 63 patients with clinically diagnosed acute adenovirus conjunctivitis attending the out-patient 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 mainly for the purpose of refraction. In the control group were 40 males and 36 females with a mean age of 35.7 (SD 17.6) years (range 8 to 60 years), and the diseased group comprised 48 males and 15 females with mean age of 30.9 (SD 11.7) years (range 5 to 63 years). The diagnosis of acute adenovirus conjunctivitis was established by detailed clinical examination including slit-lamp biomicroscopy, bacterial culture, and viral culture.

Clinically the patients presented with sudden onset of unilateral or bilateral pain, redness, and watery discharge with no visual deterioration. Ocular examination showed lid oedema, conjunctival congestion, and chemosis of bulbar conjunctiva, with frequent subconjunctival haemorrhages. There was no corneal involvement in any patient as confirmed by fluorescein staining and subsequent slit-lamp examination with cobalt blue filter.

The patients were subgrouped as mild or severe according to the clinical presentation. The patients with mild conjunctivitis had minimal lid oedema, conjunctival congestion, and no subconjunctival haemorrhage or chemosis of the bulbar conjunctiva. However, patients with severe conjunctivitis had moderate lid oedema, extensive conjunctival congestion, and chemosis of bulbar conjunctiva and subconjunctival haemorrhage. All such patients who showed any positive bacterial culture were excluded from the study.

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Specific tests adopted for the identification of adenovirus were: haemagglutination, haemagglutination inhibition, haemadsorption, complement fixation test, and serum inhibitory titre against adenovirus.^{14,15} The reference standard antigen and the antisera were obtained from the National Institutes of Health, Bethesda, USA. The typing of adenovirus was done by the numbering system proposed by Norrby.¹⁶ Adenovirus types 2, 7, or 8 could be cultured from only 26 out of 63 patients.¹³

Tear samples were also collected for α_1 -antitrypsin and serum albumin measurements from 41 patients during the remission stage, when there was no clinical evidence of conjunctivitis.

Stimulated tear samples (100 to 200 μ l) were collected by the method described previously¹⁷ and stored at -20°C until assayed. The α_1 -antitrypsin and serum albumin levels in tear fluid samples were quantified by electroimmunodiffusion.¹⁸ Mono-specific goat antihuman α_1 -antitrypsin and anti-human serum albumin sera and their respective reference standards were obtained from Meloy Laboratories, Virginia, USA. The levels of the two proteins in tear fluids were calculated from the calibration curve constructed by incorporating three known concentrations of the reference standards for every set of determinations. The results were statistically analysed by Student's *t* test.

Results

The α_1 -antitrypsin and serum albumin levels in tear fluids in patients with clinically diagnosed acute adenovirus conjunctivitis and healthy controls are given in Table 1. The mean α_1 -antitrypsin level in healthy controls was 12.1 mg/l. The mean levels in patients with acute adenovirus conjunctivitis was 47.8 mg/l for α_1 -antitrypsin and 144.5 mg/l for serum albumin in tear fluids. There was a statistically significant difference when the tear α_1 -antitrypsin ($p<0.001$) and serum albumin ($p<0.001$) levels in the

patients with conjunctivitis were compared with the respective levels in healthy controls. The tear α_1 -antitrypsin levels in healthy controls were 4.4 mg/l in males and 4.0 mg/l in females, and the difference in the two sexes was not statistically significant ($p<0.50$). Similarly serum albumin levels in tear fluids was 11.5 mg/l in males and 12.7 mg/l in females, and the difference in the two sexes was not statistically significant ($p>0.40$). In the patients with acute adenovirus conjunctivitis there was no significant difference ($p>0.50$) when the tear α_1 -antitrypsin level in males (45.3 mg/l) and females (50.3 mg/l) were compared. Similarly when the serum albumin levels in tear fluids in patients with conjunctivitis in males (141.2 mg/l) and females (151.7 mg/l) were compared, there was no significant difference ($p>0.60$).

Table 2 shows the tear α_1 -antitrypsin and serum albumin levels in mild and severe stages of clinically diagnosed acute adenovirus conjunctivitis. There were significant increases ($p<0.001$) in comparison with the respective levels in healthy controls both in mild and in severe stages for α_1 -antitrypsin and serum albumin levels in tear fluids.

The tear α_1 -antitrypsin and serum albumin levels in viral isolation-positive and viral isolation-negative patients are given in Table 3. There was a statistically significant increase ($p<0.001$) in tear α_1 -antitrypsin levels when the levels in healthy controls were compared with the levels both in viral isolation-positive and viral isolation-negative patients. However, there was no statistically significant difference ($p>0.20$) between the tear α_1 -antitrypsin level

Table 1 α_1 -Antitrypsin and serum albumin in tear fluids in healthy subjects and patients with acute adenovirus conjunctivitis (mg/l)

	No. of cases	α_1 -Antitrypsin		Serum albumin	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Healthy subjects	76	4.2 $\pm$ 2.2	1.0-10.0	12.1 $\pm$ 5.5	1.6-24.0
Male	40	4.4 $\pm$ 2.2	1.0-10.0	11.5 $\pm$ 6.0	1.6-24.0
Female	36	4.0 $\pm$ 2.1	1.0-8.8	12.7 $\pm$ 5.0	2.1-20.4
Conjunctivitis	63	48.0 $\pm$ 26.9	10.2-97.4	144.5 $\pm$ 62.1	18.6-206.8
Male	29	45.3 $\pm$ 28.4	10.8-81.7	141.2 $\pm$ 63.7	29.4-206.8
Female	34	50.3 $\pm$ 25.8	10.2-97.4	151.7 $\pm$ 58.8	18.6-206.8

Table 2 α_1 -Antitrypsin and serum albumin levels in tear fluids in mg/l in mild and severe stages of acute adenovirus conjunctivitis

	No. of cases	α_1 -Antitrypsin		Serum albumin	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Mild	25	19.4 $\pm$ 8.0	10.2-41.0	85.6 $\pm$ 60.5	18.6-171.5
Severe	38	66.8 $\pm$ 16.0	28.5-97.4	183.2 $\pm$ 14.2	141.5-206.8

Table 3 α_1 -Antitrypsin and serum albumin in tear fluids in mg/l in the acute phase in viral isolation-positive and viral isolation-negative patients with acute adenovirus conjunctivitis

	No. of cases	α_1 -Antitrypsin		Serum albumin	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Isolation-positive	26	53.7 $\pm$ 25.4	15.8-97.4	134.9 $\pm$ 71.8	18.6-196.8
Isolation-negative	37	44.0 $\pm$ 27.5	10.2-89.3	151.2 $\pm$ 54.2	29.4-206.8

Table 4 α_1 -Antitrypsin and serum albumin in tear fluids in mg/l in the acute and remission stages of acute adenovirus conjunctivitis

	No. of cases	α_1 -Antitrypsin		Serum albumin	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Acute	41	42.9 $\pm$ 26.1	11.9–80.9	141.9 $\pm$ 63.3	22.7–206.8
Remission	41	4.8 $\pm$ 1.7	1.7–8.8	12.9 $\pm$ 5.2	7.0–34.0

in viral isolation-positive patients (53.7 mg/l) and viral isolation-negative patients (44.0 mg/l). Similar results were observed in respect of serum albumin levels in tear fluids in viral isolation-negative and viral isolation-positive patients.

The tear α_1 -antitrypsin levels were measured in 41 patients during the acute and remission stages (Table 4). The mean level during the acute stage was 42.9 mg/l which decreased to 4.8 mg/l during the remission stage. There was a statistically significant difference ($p<0.001$) between the levels in the acute and remission stages. However, there was no significant difference ($p>0.20$) between the tear α_1 -antitrypsin levels in healthy subjects and in the remission stage. Similarly the level of serum albumin in tear fluids during the acute phase was 141.9 mg/l and it decreased to 12.9 mg/l during the remission stage. There was no statistically significant difference ($p>0.50$) between the level of serum albumin in tear fluids in healthy controls and in the remission stage.

Discussion

Serum α_1 -antitrypsin is frequently increased in malignancy,¹⁹ acute infections,²⁰ corticosteroid therapy,²¹ rheumatoid arthritis,²² pregnancy,²³ oestrogen therapy,²⁴ sarcoidosis,²⁵ leprosy,²⁶ and uveitis.²⁷ There is good evidence that α_1 -antitrypsin is involved in the regulation of the immune system.⁶ There are a few studies on the study of tear α_1 -antitrypsin levels in various ocular conditions. Bacterial infections of the conjunctiva and cornea cause an increase of α_1 -antitrypsin level, and the measurement of its level may help in the diagnosis of ocular inflammation.¹⁰ It has also been shown that tear α_1 -antitrypsin levels may rise in inflamed eyes in the absence of corneal involvement, for example, in cases of rosacea keratitis and allergic conjunctivitis.^{7,9} In fact in cases of allergic conjunctivitis tear levels of both α_1 -antitrypsin and serum albumin were observed to approach serum levels rapidly, indicating a direct leak from inflamed vessels.⁷

The tear α_1 -antitrypsin level in healthy controls in the present study are comparable to the levels reported by others.^{7,9} We have measured the levels in the patients with clinically diagnosed acute adeno-

virus conjunctivitis in an attempt to determine the relationship, if any, between conjunctival inflammation per se and tear α_1 -antitrypsin levels.

A remarkable increase in α_1 -antitrypsin and serum albumin was observed in tear fluids from inflamed eyes with acute adenovirus conjunctivitis. There is general agreement between the extent of conjunctival inflammation and the levels of the two proteins in tear fluids (Table 2). The levels of the two proteins in tear fluids during the remission stage fell to those found in healthy controls. It is therefore likely that the high levels of α_1 -antitrypsin and serum albumin in tear fluids in patients with acute adenovirus conjunctivitis were due to the inflammation and not inherent or pre-existent.

The origin of the proteins in tear fluids is not clear. Active transport, transudation from serum, local production, or some combination of these factors may be involved. The route(s) by which increased amounts of α_1 -antitrypsin enters tears in eyes inflamed from acute adenovirus conjunctivitis is unknown. The most likely explanation is that the α_1 -antitrypsin and serum albumin leak passively from blood vessels that supply inflamed eyes. In support of this interpretation is the observation that the ratio of serum albumin to α_1 -antitrypsin is the same in tear fluids from conjunctivitis patients with raised α_1 -antitrypsin levels as in healthy controls.

α_1 -Antitrypsin acts like an acute-phase reactant, and its level tends to rise in inflammatory conditions.²⁸ It has been commented that because of the acute reactant nature its concentration may change dramatically in the presence of inflammation.¹ The protease inhibitor may also have a protective function.²⁶ It has been suggested that during infection α_1 -antitrypsin production increases to counteract endogenous or exogenous protease.^{29,30} It is possible that there is an excessive load of proteases in tears in acute adenovirus conjunctivitis which may be counterbalanced by the release of α_1 -antitrypsin.

The raised α_1 -antitrypsin and serum albumin in tear fluids in patients with acute adenovirus conjunctivitis in the present study are probably an indicator of inflammation rather than infection, because the levels of the two proteins were markedly high in viral isolation-positive and viral isolation-negative patients. It will be interesting to study the level of these two proteins in other inflammatory diseases of the cornea and conjunctiva.

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