Immunoglobulin patterns in keratoconus with particular reference to total and specific IgE levels

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SUMMARY  A selection of sufferers from keratoconus and random controls were assessed in order to substantiate claims that there existed a significant incidence of patients with both keratoconus and a raised serum level of immunoglobulin E. The results appeared to confirm a high incidence of raised total serum IgE levels in patients with keratoconus and also indicated that the additional measurement of serum specific IgE was more sensitive than total IgE. In fact 59% of the patients with keratoconus in our study were identified as having significantly raised levels of specific immunoglobulin E, and 52% were identifiable by measuring total immunoglobulin E. This compares favourably with a previous incidence in a keratoconus population of 47% with raised total immunoglobulin E. Our study avoids clinical estimation of known associated systemic manifestations of atopic disease, thereby reaffirming the correlation between raised serum levels of IgE and keratoconus as objectively as possible. This identification pattern may be useful in reviewing high-risk groups and aid in the earlier detection of the condition.

Keratoconus is an ectasis of central corneal tissue which may also involve the periphery. Presenting in adolescence, the condition is usually self-limiting within 5-10 years, but it can be progressive or undergo an acute relapse often in the fourth decade. If the condition does not become arrested early in its development or shows an acute relapse, the patient may require surgical intervention by means of keratoplasty. On microscopic examination dehiscences are present in Bowman’s membrane and the epithelial basement membrane, and the corneal stromal lamellae are reduced in number and attenuated. If Descemet’s membrane is breached, acute oedema or corneal hydrops may be established.

Keratoconus has been loosely associated with atopic disease on many occasions.1-5 More recent reports have substantiated by immunological means the clinical impression that atopic disease, in any of its presentations, was more common in keratoconus.6 7 The known immunological disturbance associated with atopic disease, namely, raised serum levels of immunoglobulin E, was also shown to exist at a significant level in keratoconus patients.8 This observation therefore offers the possibility of an immunological marker for keratoconus. Previous studies also gave an indication, though not proved statistically, that there was a more general disturbance of autoimmunity and gammaglobulin status.9 10 These workers had detected raised IgG and IgM plus selected but nonspecific auto-antibodies in the sera of their keratoconus groups.

An indication of how diverse the results have been can be summarised as follows. On the basis of a clinical estimation of the association between atopic disease and keratoconus the incidence has varied from no correlation up to 35% of keratoconus patients having atopic disease.8 11 12 The most recent figure of 47% is based not on clinical findings but on the measurement of raised total immunoglobulin E.8 In this study a highly selective group of keratoconus patients had an incidence of 60% with raised total IgE. These were patients who had both keratoconus and a clinical expression of the atopic state. It was our intention to be entirely unselective on clinical grounds and attempt to gain a high degree of specificity from laboratory investigation alone. A group of keratoconus patients of both preoperative and postoperative types were selected at random from outpatient clinics.
In addition total and specific IgE levels in a random unselected group of the population were studied for comparison.

Materials and methods

Venepuncture samples were collected from 27 patients, 7 females and 20 males, who were diagnosed as having keratoconus. The clinical diagnosis of keratoconus was made by using the slit-lamp biomicroscope, corneal reflection patterns, and measurement of gross refractive distortion. Many clinics assisted in the collection of samples, and patients were drawn from a large geographic area, including both west and central Scotland.

After separation the samples of sera were stored at −20°C until assay. The immunoglobulins G, A, and M were measured by radial immunodiffusion technique using Behring plates. Total and specific IgE levels were measured by solid-phase radioimmunoassay. Total serum IgE was estimated by the paper radio immuno sorbent test (PRIST) as supplied by Pharmacia. Specific IgE to egg white, milk, fish (cod), house dust mite, cat epithelium, dog dander, and the grass pollen *Poa pratensis* were measured by the radio allergo sorbent technique (RAST) as supplied by Pharmacia.

Autoantibodies against cell nuclei, smooth muscle proteins, and mitochondria were looked for by immunofluorescence tests performed on frozen sections of rat stomach, liver, and kidney. Rheumatoid factor was detected by latex agglutination tests with commercial kits (Wellcome).

Blood samples were also taken from 46 normal unselected adults. The total and specific IgE levels of these control sera were measured to obtain a baseline for the incidence of raised total and specific IgE levels in a control population.

Results

Rahi *et al.* reported increased levels of immunoglobulins G and M in Keratoconus. In contrast to these findings the mean serum levels of IgG, IgA, and IgM in our group of keratoconus patients were not significantly different from the normal means as judged by a 1-sample Wilcoxon's test (Table 1). Total serum immunoglobulin E, however, was found to be significantly increased in keratoconus when compared with our control population (*p*<0.0001) by Wilcoxon's rank sum test for 2 samples (Table 2); while the incidence of positive RAST tests in these patients, 59%, was significantly higher than in the controls, 13% (Table 3) (*p*<0.001 by the x² test. In particular reference to Table 3 it will be noted that a positive RAST result is arbitrarily taken as a combined RAST screen score of >2-1 (3×0.7). This incorporates samples with one single high value or several lower values. Also tabulated is the spectrum of specific immunoglobulin E response to specific allergens (Table 4).

We found no increased incidence of antinuclear antibodies, rheumatoid factor, antimitochondrial antibodies, or antibodies to smooth muscle in comparison with the controls.

Discussion

The main feature of this study is the measurement of serum levels of specific immunoglobulin E in addition to the measurement of total immunoglobulin E. In previous work only the total IgE has been estimated, and this has produced an overall incidence of 47% of keratoconus patients having a raised level of IgE; the
The pathological process remains obscure, but at the level of incidence postulated in this study it is important to consider why there should be recordable evidence of immunoglobulin-E-mediated hypersensitivity in keratoconus sufferers. The cornea is avascular and virtually acellular under normal circumstances. Any IgE-mediated response requires previous exposure of the immune system to a sensitising allergen. No definite histological evidence exists of corneal involvement in the immune response system. The timing of the exposure or some other extraneous factor may be of importance. A further examination of the patients, their immediate relatives, and possibly their environment may be beneficial in determining potential high-risk groups. A raised serum level of IgE may in itself be a high-risk factor.

Despite the interest of the above laboratory results it was not possible to confirm previous claims of further disturbance of immunopathology. No statistically significant change in other immunoglobulins was measured. Similarly no pattern of significance was detected in estimates of the levels of selected auto-antibodies. Therefore no correlation can be postulated as in previous work, and we would be reluctant to accept a recent concept that keratoconus was a form of collagen disease expressed solely in ocular tissue. The pathology is perhaps misleading, because altered collagen appears to exist, but rather than presenting as a reaction to immunological assault it appears to be produced by abnormal keratocytes. The reason for this apparent cellular disturbance is obscure, but in considering the activities of the cellular elements in the cornea we return to the original question of aetiology in keratoconus. No direct cause and effect has been established in this study, but we have categorically confirmed the high degree of association between keratoconus and raised immunoglobulin E.

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References