

Impression cytology in Down's syndrome

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Abstract

Aim—To evaluate both the number and the average distribution of goblet cells, which are responsible for the production of the mucin layer of the tear film, in the bulbar conjunctiva of patients with Down's syndrome. Previous research had used the ferning test to indicate an alteration in Down's syndrome, but had not determined which film layer was involved.

Methods—The presence of goblet cells in the bulbar conjunctiva of 30 subjects (15 with Down's syndrome, and 15 normal control subjects) was evaluated using impression cytology.

Results—A marked reduction of goblet cells was found in the Down's syndrome group (81.4 per mm²) when compared with the control group, where (209.8 per mm²) was found.

Conclusion—The deficit observed appears to be the cause of the tear film alterations observed in Down's syndrome. In turn, this may often lead to the formation of dry spots, and to consequent frequent infections of the anterior segment of the eye. While it is further hypothesised that the alteration of the conjunctival epithelium in Down's syndrome may be due to an altered metabolism of some element or elements, such as vitamin A, further research will be necessary to corroborate this.

(*Br J Ophthalmol* 1997;81:683-685)

In a previous study on patients with Down's syndrome we have already shown that the tear film is altered, as demonstrated by the presence of abnormalities when the ferning test is used.¹ However, this test does not provide any further information as to exactly which structural element of the lacrimal secretion is involved in this pathological alteration.

For this reason, the aim of the present study was to evaluate the number of goblet cells (responsible for the production of the mucin layer) in the bulbar conjunctiva of subjects with Down's syndrome.

Subjects and methods

For this study we used the impression cytology method as described by Nelson in 1988² and introduced by Egbert *et al.*³ A round 5 mm Millepore filter was applied on the ophthalmodynamometer piston. To avoid provoking pain, we obtained conjunctival anaesthesia by using eyedrops containing 0.3% oxybuprocaine. The filter disc was mounted on the ophthalmodynamometer and gently placed on the inferior

temporal bulbar conjunctiva. We applied pressure of 70 g for 4 seconds, and the filter disc was slowly peeled off. To improve the quality of the test by increasing the peeling effect, in addition to Nelson's original method,² we engraved a series of parallel thin grooves on the surface of the filter with a surgery forceps.

The tissue samples were immediately fixed with polyethylene glycol. The filter was subsequently rehydrated with distilled water before staining with periodic acid Schiff and haematoxylin. Samples were then dehydrated through a graded series of alcohol and made transparent by immersion in xylene. Finally, the specimens were mounted on a glass slide using Eukit and were observed with a Zeiss phase contrast microscope at × 400 magnification.

Goblet cell number was estimated on a 0.04 mm² area by means of a graduated grid. The mean number in 10 randomly selected areas was obtained for each subject.

This standard method was used for 15 subjects affected by Down's syndrome with a mean age of 15 (SD 5) years (nine males and six females; 30 eyes).

The results obtained from this group were compared with those obtained from an age matched group of 15 control subjects (eight males and seven females; 30 eyes) without eye disease.

Statistical analysis of the two groups was carried out using the Student's *t* test.

Results

The average density of goblet cells in normal control subjects was 209.8 per mm² (SD 77.68); the average density of goblet cells in the group of patients with Down's syndrome was remarkably lower, 81.4 per mm² (50.88) (Table 1).

The difference found between normal control subjects and those with Down's syndrome was statistically significant (*p* < 0.001).

Moreover, in these patients the epithelial cells appeared to be smaller than in control subjects; there was a poor cytoplasm and a pyknotic nucleus (Fig 1).

The test gave largely similar results in both eyes of each patient with practically no effect of age or sex being evident.⁴

Table 1 Comparison between the number of conjunctival goblet cells found in two groups of subjects (mean age 15 (SD 5) years)

	Down's syndrome		Controls		Student's <i>t</i> test
	Mean	SD	Mean	SD	
Sex: M/F	9/6		8/7		
Goblet cells per mm ²	81.4	50.88	209.8	77.68	<i>p</i> < 0.001

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Accepted for publication
 10 April 1997

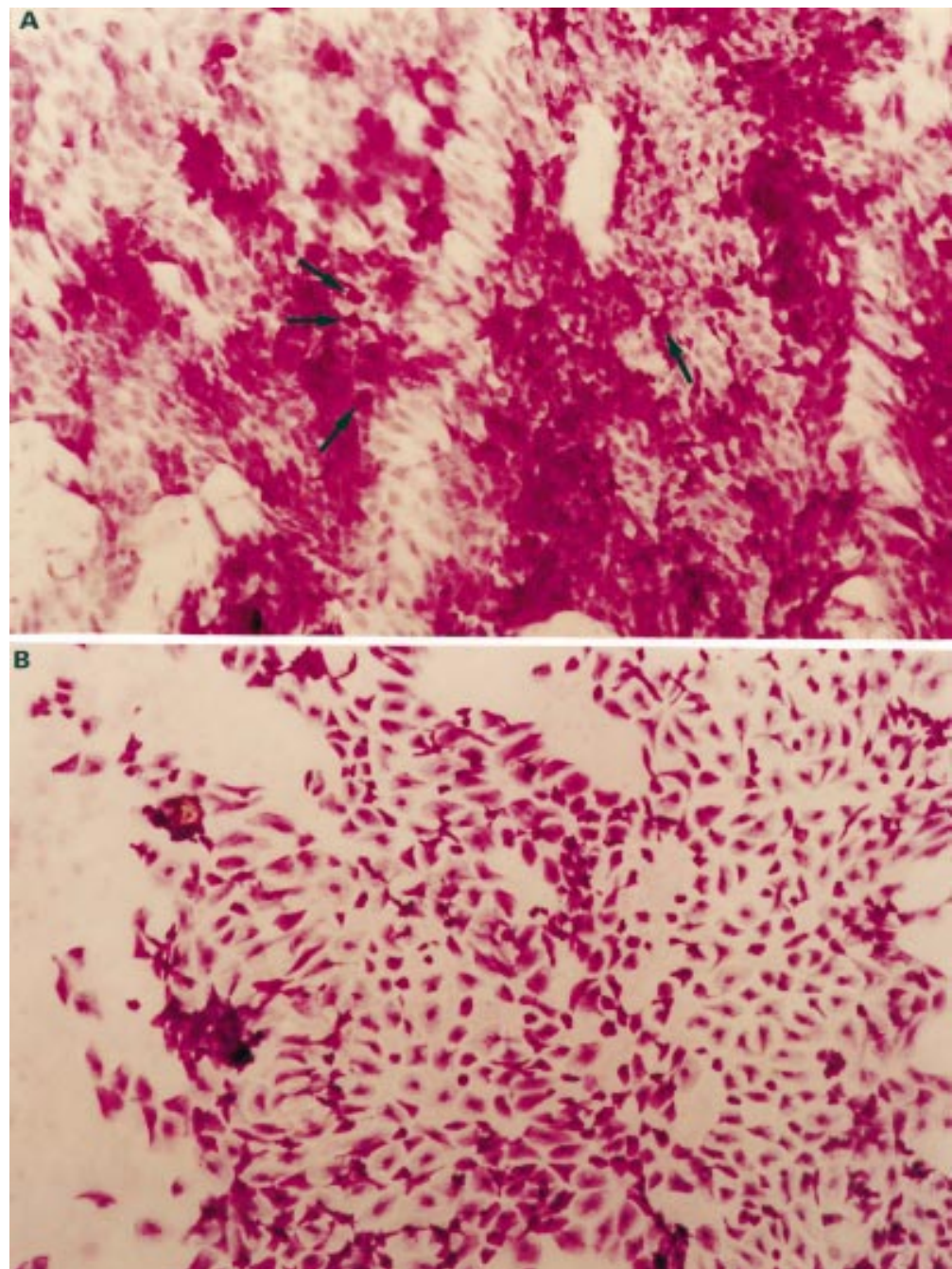


Figure 1 (A) Normal control subject: note the small round epithelial cells with large nuclei. Goblet cells (arrows) are abundant, plump, and oval with intensely periodic acid Schiff positive cytoplasm. (B) Down's syndrome subject: in this case goblet cells are completely absent; epithelial cells are reduced in size and polygonal nuclei are small and pycnotic.

Discussion

Firstly, it is important to underline that the values of goblet cell density we found in normal control subjects are very similar to those reported by other authors²⁻⁶ while the number of conjunctival goblet cells found in patients with Down's syndrome appears to be close to that already observed in other clinical conditions, such as dry keratoconjunctiva, ocular pemphigoid, and xerophthalmia.²⁻⁵⁻⁸

The ferning test had already allowed us to report alterations in the tear structure in Down's syndrome¹ and we had hypothesised that such a change might be related to an alteration in the amount of mucin produced by

the conjunctival goblet cells. The detection of a significant reduction in the number of goblet cells in patients with Down's syndrome strongly supports such a hypothesis and represents evidence of the involvement of the mucin layer in the different tear structure which we had found in these subjects. A significant reduction in the level of mucin implies a consequential rapid degeneration of the tear film—that is, the creation of hydrophobic areas on the cornea and conjunctiva, which in turn can give rise to increased risk of infection.

Additional evidence in support of this hypothesis is provided by the presence of an altered conjunctive epithelium with reduced

cell size, reduced cytoplasm, and small pyknotic nuclei (Fig 1).

The anomalous results obtained by the ferning test in subjects with Down's syndrome may be explained by the consequentially different reaction produced by a substantially reduced mucin layer and the electrolytes which are dissolved in the watery layer.

In conclusion, we are able to report a definite and substantial diminution in the mean density of goblet cells in the conjunctival epithelium in subjects with Down's syndrome.

Our working hypothesis for future research is that this alteration itself derives from an altered metabolism of some element or elements. A strong contender would seem to be vitamin A, which has been demonstrated to be essential for the differentiation and maintenance of the mucosal epithelium,^{9 10} while other studies have suggested that impression cytology represents the first simple test for the early detection of physiologically significant vitamin A deficiency.^{11 12}

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Br J Ophthalmol 1997 81: 683-685

doi: 10.1136/bjo.81.8.683

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