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CLINICAL STUDY

Impression cytology of the conjunctival epithelial cells in patients with cystic fibrosis

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Abstract

Purpose **Cystic fibrosis (CF) is the most frequent lethal autosomal recessive hereditary disorder. The disease affects all secretory epithelia, including the eye, and belongs to the group of ocular surface epithelial diseases, termed keratoconjunctivitis sicca or dry eye syndrome. The aim of this study was to evaluate goblet cell population and conjunctival epithelial morphology in patients with CF.**

Methods **A total of 20 CF patients and 20 controls underwent conjunctival impression cytology.**

Results **Impression cytology showed conjunctival squamous metaplasia and goblet cell loss in patients with CF.**

Conclusion **Reduced goblet cell numbers and squamous metaplasia may be indicative of a higher degree of epithelial damage of conjunctival epithelial cells in CF patients, and the presence of neutrophils is a strong sign for an inflammatory background of this disease.**

In view of the simple, noninvasive nature of impression cytology, this technique may prove to be an important tool for the diagnosis and monitoring of dry eye changes in CF patients.

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Keywords: impression cytology; cystic fibrosis; goblet cells; squamous metaplasia; dry eye

Introduction

Cystic fibrosis (CF) is the most frequent lethal autosomal recessive hereditary disorder in Caucasian populations and is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, whose product functions as a cyclic adenosine monophosphate-regulated chloride channel in epithelial membranes.[1,2] The absent or deficient expression of CFTR protein leads to the classic CF phenotype of raised sweat chloride, recurrent respiratory infection with bronchiectasis, and early-onset pancreatic insufficiency.[3]

It is presumed that the disease affects all secretory epithelia including the eye.[4,5] The pathogenesis of ocular changes in CF is still unknown. CF belongs to the group of ocular surface epithelial diseases, termed keratoconjunctivitis sicca (KCS) or dry eye syndrome.[4,6] The causes of dry eye are multifactorial and can be related to deficiencies in any one of the components of the ocular surface and tear film.[7] An increased understanding of the changes at the ocular surface, including alterations at the cellular level in the conjunctiva, may help explain the pathogenesis of dry eye and the subsequent clinical appearance of this potentially blinding disorder.

Conjunctival impression cytology (CIC) is a relatively simple, practical, and noninvasive, or minimally invasive technique, allowing the collection of one to three layers of cells from the bulbar conjunctival surface.[8,9] This technique is rapid and convenient and is widely performed on subjects to confirm a variety of ocular surface diseases and to monitor changes in the conjunctival surface.[10,11]

The aim of this investigation was to compare conjunctival modifications at a cellular level in impression cytology specimens from both CF patients and asymptomatic healthy individuals.

Materials and methods

Forty eyes of 20 patients with CF (9 males, 11 females) between 7 and 23 years of age

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(mean, 14.82 years) as well as 40 eyes of 20 normal subjects whose age ranged from 8 to 22 years (mean, 15.51 years; 10 males, 10 females) were recruited from the III Department of Pediatric Diseases, Medical University of Bialystok, Poland, during March 2005 through January 2006. Both groups were similar regarding age and gender characteristics. All CF patients received daily 8000 IU of vitamin A. From the time of examination, the patients were taking pancreatic enzyme supplements and oral ambroxol hydrochloride. Fifteen patients were chronically infected with pulmonary *Pseudomonas aeruginosa* and therefore on maintenance treatment with nebulized colistin sulphomethate. No patients were being treated with systemic or topical corticosteroids at the time of impression cytology. The studies were conducted according to the principles established in the Declaration of Helsinki. Informed consent was obtained from the patients or from their parents before impression cytology.

After topical anaesthesia with one drop of 0.04% oxybuprocaine, two filterstrips, 13×6.5 mm in size (polyethersulphone filters, 0.20-μm pores, Supor, Gelman Sciences, Ann Arbor, MI, USA), were applied onto the superior and superotemporal bulbar conjunctiva of both eyes without exerting any pressure.[8,12] After fixation in 96% ethanol, the specimens were stained with PAS, H&E, and using the methods of May–Grunwald–Giemsa and Feulgen. Next, the specimens were dehydrated with ascending grades of ethanol and with xylene, and finally coverslipped.

Conjunctival epithelial cells were analysed microscopically with regard to their shape, size, the nucleus-to-cytoplasm (N/C) ratio, and nuclear chromatin condensation. The specimens were assigned a grade of conjunctival epithelial squamous metaplasia according to an adaptation of Nelson's grading scheme used by Nelson[10] and De Rojas *et al*.[13]

The mean goblet cell densities were counted in five randomly selected microscopic fields, each field of 0.785 mm^2 of the analysed eye section area. The analysis of the preparations and their photographic documentation were performed with an Olympus B $\times$ 50 light microscope, with video circuit and a Pentium 120 PC with Lucia G (Nikon) software for microscope image analysis.

The statistical analysis of the results was performed using Wilcoxon's sum rank test. A level of $P \leqslant 0.05$ was considered statistically significant.

Results

The average grade of squamous metaplasia in control subjects was 0.52 ± 0.13 and in patients with CF it was 2.01 ± 0.25 ($P = 0.00013$) (Figures 1 and 2). There no was no individual with grade 2 or 3 squamous metaplasia among the controls.

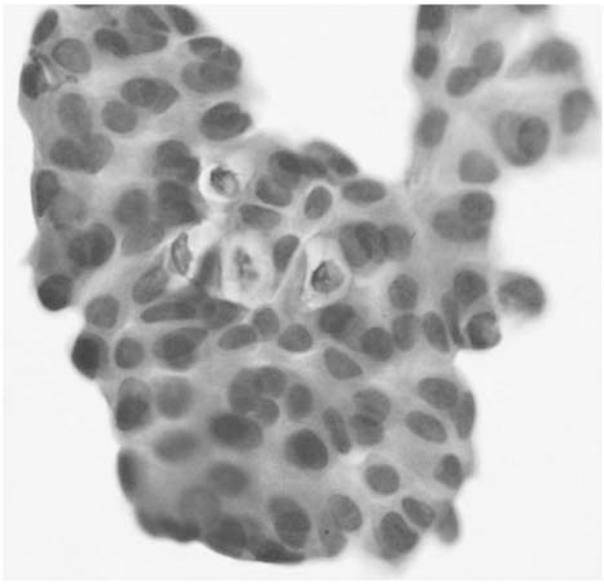

Figure 1 CIC from a normal subject: the correct picture of small, round and closely packed, contiguous epithelial cells. These cells have similar shape and sizes with eosinophilic stained cytoplasm, and numerous oval goblet cells with light cytoplasm are seen among the cells (H&E staining, $\times 40$).

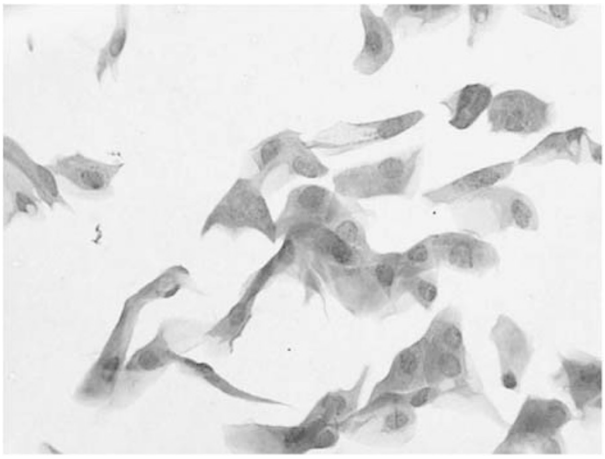

Figure 2 CIC from a patient with CF. The epithelial cells are more separated and show morphological alterations. Some of them exhibit changes in the N/C ratio, others are less regular in shape (more elongated or polygonal), and show ess eosinophilic stained cytoplasm (H&E staining, $\times 40$).

The average goblet cell densities were significantly lower in patients with CF (97 ± 55.93) when compared with those of the control group (320 ± 104.30) ($P < 0.001$).

In four patients (eight eyes) with CF, we found the presence of inflammatory cells such as mainly neutrophils (Figure 3).

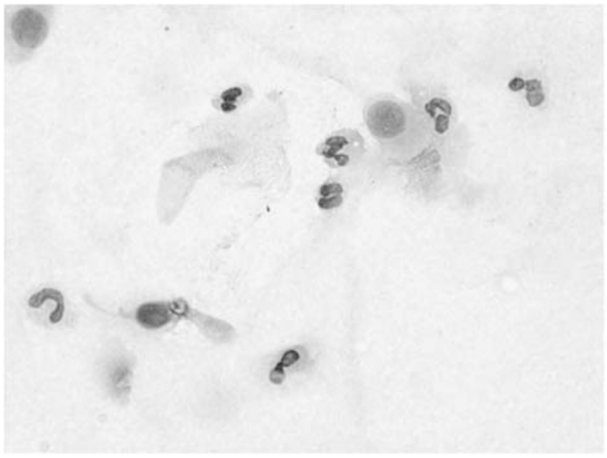

Figure 3 Conjunctival impression cytology from a patient with CF. There is the presence of neutrophils (May–Grunwald–Giemsa staining, $\times 200$).

Discussion

In this study, we investigated the morphological changes in conjunctival epithelial cells in CF patients. We performed CIC to find clues for dry eye. Impression cytology provided useful information about the nature of the ocular changes in CF patients, which was characterized by goblet cell loss and squamous metaplasia similar to other dry eye disorders.[14-16] Squamous metaplasia is a transition from a non-keratinized, stratified (secretory or non-secretory) epithelium (such as conjunctival or corneal) to a non-secretory keratinized epithelium. During squamous metaplasia of the conjunctival epithelium, there is a continuous spectrum of changes, with an early decrease and eventual loss of goblet cells and progressive morphological changes of non-goblet epithelial cells such as increased stratification and keratinization. Round blue cells with N/C ratios of 1/1 transform into more elongated and flattened (squamoid) cells with metachromatic changes of the cytoplasm (pinkish colour), and N/C ratios increasing up to 1/8 and becoming pyknotic.[8] As noted, these changes occur mainly in dry eye-related conditions.[14–17]

In our patients, we also found the presence of inflammatory cells. It is known that impression cytology has made an important contribution to the understanding of dry eye as an immune-based inflammatory condition.[18] Pflugfelder *et al*[19] showed that over 60% of the inflammatory cells seen in the inferior fornix of Sjögren's syndrome patients were T lymphocytes. Conjunctival epithelium from dry eyes harvested by impression cytology has been shown to overexpress inflammatory markers such as HLA-DR, ICAM-1, the low-affinity receptor for IgE CD23, CD40-CD440L, or Fas and APO2.7 levels by immunocytochemistry and flow cytometry.[12,20–25] Several previous studies have shown higher levels of ICAM-1 and many proinflammatory cytokines in the conjunctival epithelium from Sjögren's syndrome patients, analysed by immunofluorescence, RT-PCR, and ELISA.[19,26,27]

In summary, our findings of less goblet cell numbers and squamous metaplasia may be indicative of a higher degree of epithelial damage of conjunctival epithelial cells in CF patients, and the presence of neutrophils is a strong indicator of an inflammatory aetiology for this disease.

In view of the simple, noninvasive nature of impression cytology, this technique may prove to be an important tool for the diagnosis and monitoring of dry eye changes in patients with CF.

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