

# Conjunctival impression cytology and correlation with vitamin A levels in children with Down Syndrome\*

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## SUMMARY

**Background:** The purpose of this study was to evaluate bulbar surface epithelium changes with conjunctival impression cytology (CIC), and to determine if we could detect ocular surface abnormalities in the eyes and to correlate with serum vitamin A levels in Down syndrome (DS) patients.

**Material and Methods:** The study groups included 36 eyes of 18 DS patients who were followed up at pediatrics clinics of our hospital and 40 eyes of 20 healthy control subjects from the same age group. Specimens for CIC were obtained with a cellulose acetate filter paper from the upper bulbar conjunctiva and fixed with 70 % ethyl alcohol, 37 % formaldehyde and 20:1:1 glacial acetic acid solution. Specimens were stained with periodic acid schiff's and hematoxylin-eosin. Ocular surface changes were evaluated on the cell content of the surface conjunctival epithelium by CIC with light microscopy. Serum vitamin A level was measured with HPLC in DS patients.

**Results:** A marked reduction of goblet cell density was found in the DS group ( $73.2 \pm 21.2 \text{ mm}^2$ ) in comparison with the control group ( $113.5 \pm 30.1 \text{ mm}^2$ ) ( $p < 0.01$ ). Serum vitamin A levels were found  $1.32 \pm 0.6 \text{ mmol/l}$  in DS patients. The number of goblet cell density and vitamin A levels were evaluated and positive correlation was found in DS patients ( $p < 0.05$ ).

**Conclusion:** We have shown decreased mean density of goblet cells in patients with DS. The results indicate that CIC may be a first step simple, objective, and diagnostic test as a screening tool for early eye problems in DS. In patients with DS, ocular surface abnormalities may be prevented by early vitamin A treatment.

**Key words:** Impression cytology, eye, vitamin A, Down Syndrome

## ÖZET

### Down Sendromlu çocuklarda konjunktiva impresyon sitolojisi ve vitamin A düzeyleri ile ilişkisi

**Amaç:** Down sendromlu (DS) hastalarda bulbar yüzey epitel değişikliklerini konjunktival impresyon sitolojisi tekniği ile saptamak ve serum vitamin A düzeyi ile korelasyonunu araştırmak

**Gereç ve Yöntem:** Hasta grubunu, hastanemiz pediatri kliniğinde takip edilen 18 DS'lu hasta (36 göz), kontrol grubunu ise aynı yaş grubundaki 20 sağlıklı çocuk (40 göz) oluşturdu. Üst bulbar konjunktivaya uygulanan impresyon sitolojisi yöntemi ile sellüloz asetat kağıdı yüzeyine alınan örnekler (% 70'lik etil alkol, % 37'lik formaldehit ve glisial asetik asitin 20:1:1 oranındaki) solüsyonunda fikse edildi. Örnekler Periodik Asid Schiff ve Hematoksilin-eosin ile boyandı. Işık mikroskopisi ile oluşan okuler yüzey değişiklikleri değerlendirildi. DS hastalarda serum vitamin A düzeyi HPLC ile ölçüldü.

**Bulgular:** DS'lu grupta goblet hücre yoğunluğu ( $73.2 \pm 21.2 \text{ mm}^2$ ) kontrol grubuna göre ( $113.5 \pm 30.1 \text{ mm}^2$ ) azalmış olarak saptandı ( $p < 0.01$ ). DS'lu grupta serum vitamin A düzeyi  $1.32 \pm 0.6 \text{ mmol/l}$  olarak saptandı. DS'lu grupta goblet hücre yoğunluğu ve vitamin A düzeyi arasında pozitif korelasyon saptandı ( $p < 0.05$ ).

**Sonuç:** DS'lu hastalarda ortalama goblet hücre yoğunluğunun azaldığını gösterdik. Bu sonuç, konjunktival impresyon sitolojisinin DS'lu hastalarda erken göz problemlerinin tanısında basit, objektif, tanusal tarayıcı olarak kullanılabileceğini göstermektedir. Yine DS'lu hastalarda okuler yüzey anormallikleri erken vitamin A desteği ile önlenbilir.

**Anahtar kelimeler:** İmpresyon sitolojisi, göz, vitamin A, Down Sendromu

Down syndrome (DS), also known as Trisomy 21, is the most common genetic disorder. Its common features include poor muscle tone, short stature, a

small nose with flat nasal bridge, dry skin, immune-system suppression, developmental delays, speech difficulties, mental retardation and

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epicanthal folds. The other ocular findings in patients with DS include the following: blepharitis, conjunctivitis, prominent epicanthal folds, upward slanting of palpebral fissures, nasolacrimal duct obstruction, chronic external infections, high refractive errors, strabismus, nystagmus, keratoconus, keratoglobus, Brushfield spots, cataracts, glaucoma, and retinovascular anomalies <sup>(1)</sup>. In this situation, DS patients should go on routine ophthalmologic examination.

Vitamin A (as retinol) and its derivatives are required for the normal growth and differentiation of epithelium both in vivo and in vitro <sup>(2,3)</sup>. Systemic vitamin A depletion results in keratinization and drying of the epithelium in the gastrointestinal tract, respiratory tract, and ocular surface <sup>(4)</sup>. In the eye, vitamin A deficiency is reported to be associated not only with keratinization and squamous metaplasia but also with a reduction in the goblet cell population and an increase in conjunctival epithelial cell mitosis <sup>(5,6)</sup>. Vitamin A is of particular interest in DS because there are studies suggesting a deficiency of vitamin A in these patients <sup>(7)</sup>. However, some others report no evidence of vitamin A deficiency <sup>(8,9)</sup>.

Conjunctival impression cytology (CIC) is the technique of collection of the most superficial layers of the ocular surface by applying different collecting devices (usually filter papers) so that cells adherent to that surface are subsequently removed from the tissue and further processed for a diversity of techniques <sup>(10)</sup>. It is fast, noninvasive, easy to perform, and economical <sup>(11)</sup>. There are few studies investigating the ocular-surface changes by CIC in patients with DS <sup>(12)</sup>. Filipello et al, have suggested a hypothesis that the alterations of ocular-surface 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 while other studies have suggested that CIC represents the first simple test for the early detection of

physiologically significant vitamin A deficiency <sup>(12-14)</sup>.

In this study, ocular-surface changes including goblet cell density was evaluated by CIC and correlation with vitamin A levels was made in patients with DS who did not have lesions over the eyelids. This is the second study in which ocular surface changes in DS patients are examined by CIC and we didn't find any MEDLINE search of the English-language medical literature about correlation between plasma levels of vitamin A and ocular surface changes in DS patients.

## MATERIAL and METHODS

This prospective study was performed in the Department of Pediatrics, Histology and Ophthalmology Clinics of our Hospital. Our study group comprised of eyes 36 of 18 patients with DS (12 male, 6 female). Control group consisted of 20 age and sex matched (12 male, 8 female) healthy volunteers (40 eyes) without any abnormality on routine ophthalmological examination. The median age was  $6.2 \pm 2.9$  (range 2-14 yr) in patients with DS and  $7.9 \pm 3.2$  yr (range 2-15 yr) in the control group ( $p > 0.05$ ).

The specimens were collected from the upper bulbar conjunctiva, 5 mm from the limbus with 0.4 % oxibupracaine hydrochlorid topical anesthesia. Specimens for CIC were obtained with a cellulose acetate filter paper of 0.020  $\mu$ m pore size (Sartorius 11107-50-N) from the superior bulbar conjunctiva of both eyes of each subject by using the method that Egbert et al. described <sup>(10,15)</sup>. The cellulose acetate filters were firstly cut to obtain pieces of 3x4 mm size. The mat surface of these papers was gently pressed on the upper bulbar conjunctiva at 12:00 o'clock direction for a period of 3-4 seconds, in such that the lower edge of the paper was kept 2 mm away from the limbus. The paper was then removed slowly from the conjunctiva, while keeping the conjunctiva epithelium samples obtained at cytological level on the upper

surface of the paper. They were further fixed with 70 % ethyl alcohol, 37 % formaldehyde and 20:1:1 glacial acetic acid solution in an effort to dye, and then stored at a temperature of +4°C to avoid the evaporation of alcoholic substances (15,16). Utmost care was taken to place the samples in their correct eye group during all these procedures. Conjunctival impression cytology specimens were stained with Periodic Acid Schiff (PAS), Hematoxylin-eosin (H&E) and examined light microscope. Goblet cell number was estimated on a 0.04 mm<sup>2</sup> area by means of a graduated grid. The mean number in 10 randomly selected areas was obtained for each subject.

Measurement of vitamin A: Serum vitamin A level was measured with high-performance liquid chromatography (HPLC), LC10A series used Recipe kits (Munich/Germany).

Statistical analysis: Data were evaluated by SPSS computer package (SPSS Inc., Chicago). Values were expressed as mean±SD. A p-value <0.05 was considered as statistically significant. The Student-t test was performed to compare the patients and control group. The value and the direction of the relation between variables were investigated using Pearson and Spearman correlation coefficients.

## RESULTS

We have investigated 36 eyes of the 18 patients with DS and 40 eyes of the 20 children in the control group. The count density of goblet cells in the group of children with DS was 73.2±21.2 mm<sup>2</sup>; The count density of goblet cells in normal control subjects was 113.5±30.1 mm<sup>2</sup> (Table 1). The goblet cells population in the group of children with

DS was profoundly decreased. There was a statistically significant difference between patients with DS and the control group (p<0.01) (Figure 1 and 2). We didn't find any statistically significant difference between the right or left eye in each indivi-

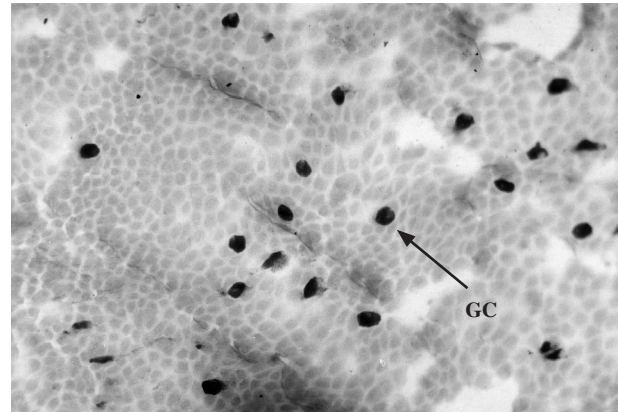


Figure 1. CIC in control group: Goblet cells (GC) are abundant, oval and plump (PAS- Hematoxylin, original magnification X 82).

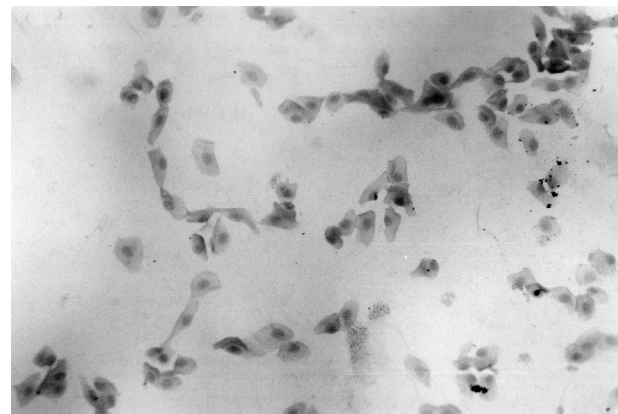


Figure 2. CIC in patients with DS; Goblet cells are absent (PAS- Hematoxylin, original magnification X 82).



Figure 3. CIC in patients with DS; Epithelial cells are reduced in size and nuclei are pycnotic (PAS- Hematoxylin, original magnification X 164).

Table 1. The count density of goblet cells in the control and DS group.

	N	mean	SD
Down Syndrome	38	73.2	21.2
Control Group	40	113.5	30.1
Statistic	p<0.01		

dual. Moreover, in these patients the epithelial cells appeared to be smaller than in control group; there was a poor cytoplasm and a pyknotic nucleus (Figure 3). Serum vitamin A levels were found to be  $1.32 \pm 0.6$   $\mu\text{mol/l}$  in the patients group. The number of goblet cell density and vitamin A levels were evaluated and positive correlation was found in DS patients ( $p < 0.05$ ).

## DISCUSSION

The ocular surface made up of stratified, nonkeratinizing cell layers, is covered by the tear film, which lubricates, hydrates, and protects the underlying epithelium. The innermost component of the tear film is mucus, composed mainly of secreted mucins, which are produced by conjunctival goblet cells, with potential additional contribution of membrane-spanning mucins expressed by corneal and conjunctival epithelia (6,17). Previous *in vitro* studies have indicated that vitamin A derivatives are required for maintaining mucosal cell differentiation, mucin production, and mucin gene expression (6,18). Vitamin A deficiency is reported to be associated reduction in the goblet cell population and an increase in conjunctival epithelial cell mitosis in the eye (5,6). Wolbach et al. have demonstrated that vitamin A is essential for the normal differentiation and survival of the mucosal epithelium, and that its deficiency causes a metaplasia of mucosal epithelium to keratinized squamous epithelium (13). The tear quantity and quality are normal despite the formation of a real metaplasia in conjunctiva is a vitamin A deficiency (16).

Supplementation with both single vitamins and vitamin mixtures have been studied in DS patients. Although sporadic reports of vitamin deficiencies have been published (7,19), most studies reveal none (9,20), and many studies have found that vitamin supplementation with either Recommended Dietary Allowance (RDA) doses or megadoses have no effect on mental ability or behavior (21,22). The present supplements tend to use RDA values, although levels of vitamin A high enough to be toxic are still

occasionally promoted. Vitamin A gastrointestinal absorption in patients with DS decreased but Pueschel et al. do not support previous reports of significantly decreased vitamin A absorption in patients with DS (9).

Impression cytology of the conjunctiva is an important diagnostic tool in investigating ocular surface disorders. Ocular surface investigations with CIC in DS patients have revealed a significantly higher rate of squamous metaplasia than in normal individuals. This difference is thought to arise from a metabolic change in elements such as vitamin A (12). The goblet cell content of the conjunctiva is a sensitive indicator of primary ocular surface disease (23). In our present study decreased goblet cell density was found in DS patients in comparison with the control group, similar to those reported by Filippello et al (12). And we were found positive correlation between plasma levels of vitamin A and the number of goblet cell density in DS patients. Filippello et al. have reported alterations in the tear structure in DS and hypothesised that such a change might be related to an alteration in the amount of mucin produced by the conjunctival goblet cells (24). The detection of a significant reduction in the number of goblet cells in patients with DS strongly supports such a hypothesis and represents evidence of the involvement of the mucin layer in the different tear structure 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 (12). The anomalous results obtained by the ferning test in subjects with DS 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 (12).

We are able to report a definite and substantial diminution in the mean density of goblet cells in the conjunctival epithelium in patients with DS. The results indicate that CIC may be a first step

simple, objective, diagnostic test, potentially useful as a screening tool for ophthalmologic examination in DS patients. Serum vitamin A is positively associated with mean density of goblet cells in the conjunctival epithelium in patients with DS. In the light of these findings, the squamous metaplasia detected in patients with DS might have resulted from vitamin A deficiency and poor nutritional stores. Thus, treatment with vitamin A can be provided in patients with DS.

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