Original article

Conjunctival impression cytology in computer users

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Abstract

Introduction: It is known that the computer users develop the features of dry eye. **Objective:** To study the cytological changes in the conjunctiva using conjunctival impression cytology in computer users and a control group. **Materials and methods:** Fifteen eyes of computer users who had used computers for more than one year and ten eyes of an age-and-sex matched control group (those who had not used computers) were studied by conjunctival impression cytology. **Results:** Conjunctival impression cytology (CIC) results in the control group were of stage 0 and stage I while the computer user group showed CIC results between stages II to stage IV. Among the computer users, the majority (> 90 %) showed stage III and stage IV changes. **Conclusion:** We found that those who used computers daily for long hours developed more CIC changes than those who worked at the computer for a shorter daily duration.

Key-words: conjunctival impression cytology, computer users, dry eye

Introduction

We are living in the computer era and computer vision syndrome is now a well-known disease entity. It is common knowledge that when we work on computers, our blinking rate goes down to such a low level that the pre-corneal tear film becomes defective. The human tear film becomes unstable after a few seconds, but it is regenerated by frequent blinking. And the majority of computer users work in an air-conditioned atmosphere which decreases the moisture percentage of the air. All these factors alter the structure of the tear film and the rate of evaporation. These lead to commonly seen problems in frequent computer users, eg, headaches, blurred vision, neck pain, redness in the eyes, fatigue and irritated eyes.

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Egbert et al introduced conjunctival impression cytology (CIC), a simple non-surgical technique, to study the different cytological responses of the conjunctiva towards various stimuli or conditions. It is a non-invasive and painless technique (Simon et al, 2002). It can be used safely in computer users without affecting their normal routine.

Conjunctival epithelial changes have been reported in both symptomatic and asymptomatic lens wearers. The main changes are squamous metaplasia, decrease in goblet cell density and presence of abnormal chromatin material in epithelial cell nuclei (Simon et al, 2002).

The purpose of this study was to evaluate the conjunctival surface changes in computer users using conjunctival impression cytology so that the severity of the effect of computers on the eye can be diagnosed in sub clinical and relatively asymptomatic stage and colossal damage to the epithelium of conjunctiva can be prevented.

Materials and methods

Fifteen eyes of computer users who had used computers for more than one year and ten eyes of age and sex-matched subjects of a control group (those who have not used computers) were studied by conjunctival impression cytology. The subjects with any past history of ocular surface disorders, ocular surgery, ocular trauma, long-term use of topical medication and those with evidence of any ocular surface disorder on slit-lamp examination were excluded. Conjunctival impressions of the cases were taken in the ophthalmology OPD of Subharti Medical College, Meerut (UP) and the cytological examination was done in the pathology department.

Cellulose acetate filter paper (Sartorius stedim biotech 11107-13-N) was cut into small squares of 5 x 5 mm. The conjunctiva was anaesthetized by topical 4% xylocaine and the filter paper was applied to the superior bulbar conjunctiva, 2 mm away from the limbus, using forceps. Gentle pressure was applied with the blunt end of the forceps for 3 - 5 seconds and then the filter paper was removed with a peeling motion. Two such samples were taken, one from the 11 o' clock and the other from the 1 o'clock position. One impression of the filter paper on a glass slide was then fixed in a solution of 70% ethyl alcohol, 37% formaldehyde and glacial acetic acid in a recommended standard volume ratio of 20:1:1 for 10 minutes and the other impression of the filter paper on glass slide in 95% ethyl alcohol. The impression of the filter paper on the glass slide fixed in 95% ethyl alcohol was stained with Papanicolaou stain and the other impression with PAS and haematoxylin stain. Both slides were then cleared in xylene and viewed under a light microscope. The morphologic changes in epithelium cells, characteristics of nuclear chromatin and goblet cell loss were noted. The entire slides were examined to determine the nature of the cytological changes.

Staging of conjunctival squamous metaplasia was done according to the systems described by Natadisastra et al (1987) as given below. CIC is considered normal if any area of the specimen demonstrated a substantial proportion of normal epithelium with evidence of goblet cells, ie, stage 0 and 1.

Stage 0 – Abundant goblet cell and mucin spots, small epithelial cells (Fig-I)

Stage 1 – Fewer goblet cells & mucin spots, small epithelial cells (Fig-II)

Stage 2 – Loss of goblet cells and mucin spots, enlarging epithelial cells

Stage 3 – Enlarging or separating epithelial cells (Fig-III)

Stage 4 – Large separate epithelial cells with scattered keratinisation, pyknotic nuclei.

Stage 5 – Large keratinised epithelial cells with pyknotic nuclei or loss of nuclei.

Results

A detailed history was taken with special reference to computer use (duration of computer use, average daily time of computer use and any features of discomfort).

Table I: Age distribution

Age group	control	computer user
20 - 30 years	6	10
> 30 years	4	5
Mean age	30.1 years	28.1 years

The study comprised of 10 eyes of the subjects of the control group of age between 20 to 45 years with mean age of 30.1 years. Among 15 computer users all were between 20 years to 42 years with mean age of 28.1 years (Table-I). X^2 test statistic was applied to find the association between the control subjects and the computer users for different age groups. A highly significant association was present between the two groups ($P < 0.001$).

Table II: Conjunctival impression cytology results

1. **Control group**	**stage 0**	**stage I**	**stage II**	**stage III**	**stage IV**
A. 20 - 30 years	3	3	-	-	-
B. > 30 years	3	1	-	-	-
2. **Computer users**					
A. 20 - 30 years	-	-	-	6	3
B. > 30 years	-	-	2	-	4

The conjunctival impression cytology (CIC) results in the control group were of stage 0 and stage I**.** The computer users showed CIC results between stages II to stage IV (Table II). Further, conjunctival impression cytology was statistically significant among the different stages of two age groups of control and computer users separately. However, a significant difference was present between the groups of the control subjects and the computer users for different stages in two age - groups (P< 0.001).

Table III A: Appearance of CIC changes with duration of computer use (hours per day)

CIC changes	0 - 4 hrs/day	4 - 6 hrs/day	> 6 hrs/day
Stage 0	-	-	-
Stage I	-	-	-
Stage II	2	-	-
Stage III	1	4	1
Stage IV	-	5	2
Stage V	-	-	-

Subjects who used computers for less than four hours per day, showed mild CIC changes, ie, stage II and III, in comparison to those subjects who used computers for more than four hours per day who showed moderate changes, ie, stage III and IV (Table III A)

Table III B: Appearance of CIC changes with duration of computer use (numbers of years)

CIC changes	< 3 Years	3 - 6 Years	> 6 Years
Stage 0	-	-	-
Stage I	-	-	-
Stage II	-	-	2
Stage III	-	4	2
Stage IV	1	2	4
Stage V	-	-	-

The duration of the computer use in terms of the number of years showed variable effect in CIC results. One subject who used a computer for less than 3 years showed stage IV CIC changes, while two subjects who used a computer for more than 6 years showed only stage II CIC changes (Table III B). The appearance of CIC changes with duration of computer use (in hrs/day) was statistically significant in different stages but not significant (P > 0.001) with duration of computer use (in years) at 0 .001 level of significance.

Cytological changes frequently seen in different stages are as follows.

Stage II PAS stained smears showed few small epithelial cells with occasional enlarging polyhedral cells. No goblet cell or mucin spots were seen. Papanicolaou stained smear revealed polyhedral cells with a thick cytoplasm. No keratinization was observed.

Stage III smears showed a single cluster of stratified squamous cells and occasionally anucleate squamous cells. No columnar epithelial cells were seen but occasionally spindle shaped cells were present. PAS staining also did not reveal any mucus or goblet cell.

Stage IV smears were usually paucicellular and showed anucleate squamous and discrete nucleated squamous cells. There was a complete absence of mucin and goblet cells. The PAS stained smears showed occasional discrete polyhedral cells exhibiting thick cytoplasm with focal keratinization.

Discussion

Impression cytology (IC) allows cells to be harvested from the ocular surface noninvasively. The superficial layers of the epithelium are removed by application of cellulose acetate filters or biopore membranes, and the cells can be subsequently analyzed by various methods, depending on the objective of the investigation or pathology involved. IC techniques are easily learned, can be performed in an outpatient setting, and cause virtually no discomfort to the patient. IC facilitates the diagnosis of ocular surface disorders, including, among others, keratoconjunctivitis sicca, ocular surface squamous neoplasia, and ocular surface infections. During the past decade, IC has been used increasingly to assist in the diagnosis of ocular surface diseases and improving our understanding of their pathophysiology, and provide biomarkers to be used as outcome measures in clinical trials. Dry eye

disease is one area in which IC has contributed to significant advances (Lopin et al, 2007).

Dry eye is a complex clinicopathological entity involving tear film, lacrimal glands, eyelids, and a wide spectrum of ocular surface cells, including epithelial, inflammatory, immune, and goblet cells. From the tightly regulated lacrimal film functions and structure, a large variety of investigations have been developed, including tear meniscus measurements, fluorophotometry, meibometry, interference pattern analysis, evaporation rate, tear osmolarity, and thermography. Dry eye conditions also interfere with the ocular surface, causing corneal irregularities that may be explored using the techniques of videokeratography and in vivo confocal microscopy, or optical impairment, as confirmed by aberrometry. At the level of ocular surface cells, impression cytology remains a standard for assessing cell alterations (Labbe et al, 2007).

Shreshtha et al (2011) have done a case control study including consecutive cases of dry eye syndrome. Individuals without dry eye were taken as control. Impression of conjunctiva with cellulose acetate filter paper was taken from the infero-nasal bulbar conjunctiva and was stained with Periodic Acid- Schiff (PAS) and counter-stained with haematoxylin and eosin. Goblet cell density was found to be significantly reduced in dry eye syndrome. Hence, the impression cytology was considered a useful test for the diagnosis of dry eye syndrome.

It is well known that there is a lack of association between symptoms and signs in patients with dry eye disease. Morales-Ferandez et al (2010) had done a study to assess if there was any agreement between common dry eye diagnostic tests and compare them with impression cytology. The examination included: the quality of life test ocular surface disease index (OSDI), break up time test (BUT), fluorescein staining, Schirmer's test and finally the study of goblet and conjunctival epithelial cells with impression cytology. The conclusion of the study was that the impression cytology is a valuable test in the diagnosis of dry eye. Although the number of goblet cells is not a sufficient diagnostic criterion, its joint assessment with the grade of metaplasia and the other dry eye tests is useful.

Fiqueroa-ortiz et al (2011) have done a study to investigate whether different grades of diabetic retinopathy correlate with the tear and ocular surface function in diabetic patients without subjective symptoms of dry eye. The tear film and ocular surface were evaluated using the Schirmer test, tear film break-up time (BUT), fluore-scein and lissamine green staining test, and conjunctival impression cytology. The diabetic retinopathy patients without subjective symptoms of dry eye and normal Schirmer and BUT tests showed pathological grades of squamous metaplasia.

The ocular surface is a frequent target tissue of mucosal chronic chronic graft verses host disease (cGVHD). Tatematsu et al (2011) investigated the histopathological features of the conjunctival microvilli in patients with cGVHD. Conjunctival tissue specimens from patients with cGVHD or Sjögren's syndrome (SS) or from healthy individuals were examined by light microscopy, impression cytology, and immunohistochemistry. The cGVHD conjunctivae showed significantly more metaplasia and fewer goblet cells than the SS and normal conjunctivae.

Conjunctival epithelial changes have been reported in both symptomatic and non -symptomatic lens wearers. The main changes are squamous metaplasia, decrease in goblet cell density and presence of abnormal chromatin material in epithelial cell nuclei.

Conjunctival impression cytology, used in our study, is a non-invasive and a safe technique. It demonstrates the epithelial cell morphology, nuclear cytoplasmic characteristics, goblet cell density and presence or absence of inflammatory cells. There was no need to discontinue computer work as in conjunctival biopsy procedures, which is an invasive technique.

In the literature, we did not find any study related to the effect of computer use on the conjunctival impression cytology.

In our study, 10 control subjects and 15 computer users were included having the mean age of 30.1 and 28.1years respectively. The control group showed stage O to stage I changes on CIC results, which are normal variation according to staging system described by Natdisastra et al (1987).

Among the computer users group, 13 subjects showed stage III and stage IV changes on CIC results. However, the two subjects from the computer users' group showed only stage II CIC changes although they had been using a computer for more than six years, but their daily computer use hours were less than four hours per day. In our view, this is a significant finding which shows that if our computer use period is less than four hours per day, it will not affect the cytology of the conjunctiva as adversely as when we use the computer for more than four hours per day. This is probably due to the time available for conjunctival epithelium to repair its defect during the period when the subject was not working on the computer. In contrast to this, one subject showed stage IV changes even when he was using the computer for less than three years, but his daily sitting at a computer was more than six hours per day.

Conclusion

This showed that a person who uses computer daily for long hours develops more CIC changes than those who have a short duration of computer work. Although the sample size in our study is small, the findings are very significant. More detailed and comprehensive clinical studies to evaluate the effect of computer use on the ocular surface are necessary.

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