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Alterations of the tear film and ocular surface health in chronic smokers

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CLINICAL STUDY

Abstract

Objective   To investigate the effects of chronic smoking on ocular surface and tear functions.

Methods   Fifteen right eyes of 15 healthy chronic smokers (9 men, 6 women; age range: 36–47 years) who smoked 20 cigarettes per day for 20 years and 20 eyes of 20 control non-smokers (12 men, 8 women; age range: 38–43 years) were included in this prospective study. All subjects underwent measurements of breath and haemoglobin CO concentration, tear lipid layer interferometry, evaporimetry, tear film break-up time (TBUT), Schirmer's I test, corneal fluorescein staining, conjunctival impression, and brush cytology.

Results   The mean Hb CO level was significantly higher in smokers compared to non-smokers. TBUT was also significantly shorter in smokers. Tear lipid layer showed significant slowing in spread over the tear film with a concomitant significant increase in tear evaporation rate. Conjunctival impression cytology revealed significant loss of goblet cells and squamous metaplasia in smokers. Brush cytology showed significant conjunctival neutrophil infiltration in smoker subjects.

Conclusion   Chronic smoking induced distinctive quantitative and qualitative disturbances on the ocular surface health.

Eye (2008) **22**, 961–968; doi:10.1038/eye.2008.78; published online 18 April 2008

Introduction

Cigarette smoke is an essential source of intake of heavy metals and toxic mineral elements, all known to be poisonous in high concentrations.[1] These toxic compounds have multiple adverse physiologic effects with profound pathological disturbances in different organs, including the eye. Fume particles and noxious gases such as nicotine and carbon monoxide may influence the circulatory system by inducing profound vasospasm, platelet aggregation, or leading to oxidative damage to lipids, proteins, and cellular DNA.[2–8]

Epidemiological data link cigarette smoking as a risk factor for many ophthalmological disorders including strabismus in offspring of pregnant smokers, anterior ischaemic optic neuropathy, age related macular degeneration, cataract, thyroid ophthalmopathy, primary open angle glaucoma, diabetic retinopathy, uveal melanoma, conjunctival intraepithelial neoplasia, ocular sarcoidosis, and development of dry eyes.[9–26] The Beaver Dam Study related current active smoking and past history of smoking to a higher prevalence of dry eye disease.[27] The Blue Mountain Eye Study also recognized smoking as an important risk factor for dry eyes.[28] Demographic surveys have shown that 25–40% of employees experience cigarette smoke in their work environment and that 25% of them have dry eye related symptoms.[5] Despite the availability of important epidemiological data on hazardous effects of cigarette smoke, there are surprisingly only a few studies related to the alterations of the ocular surface and tear functions in cigarette smokers.

In this study, we investigated the effects of long term exposure to cigarette smoke on the tear film and ocular surface health of in chronic healthy smokers and compared the results with those of non-smoker subjects.

Materials and methods

Fifteen eyes of 15 chronic healthy smokers (9 men, 6 women; age range: 36–47 years) and 20

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eyes of 20 control non-smokers (12 men, 8 women; age range: 38–43 years) were included in this prospective study. Subjects who smoked 20 cigarettes per day for 20 years were included as chronic smokers according to the protocol of the study. Passive smoking status was determined by asking the control subjects about the presence of smoking history among household members or at work. Control subjects with a history of passive cigarette-smoke exposure or who spent substantial time in a smoky atmosphere at home or at work within the last 6 months were excluded from the current study. Smoking status was evaluated by calculating the pack years of cigarette smoking (the average number of cigarettes per day times the number of years of smoking) which served as a measure of cumulative exposure.[29] At the time of enrolment and acquisition of informed consents when the appointments for CO measurements and tear function tests were made, smokers were requested not to smoke for at least 6 h before their examinations on the morning of the testings for breath and haemoglobin CO concentrations. Examination procedures were board reviewed and informed consent was obtained from all subjects.

Examinations and study exclusion criteria

Subjects with contact lenses, ocular diseases including meibomian gland dysfunction, blepharitis, infectious keratoconjunctivitis, systemic diseases with known associations to ocular surface diseases were excluded. Subjects who were diagnosed as having dry eyes according to the diagnostic criteria of the Dry eye Research Group in Japan, were also excluded from the study.[30]

Ocular symptoms

All subjects were asked about the presence of ocular symptoms and to state their worst symptom. Smokers were asked if their worst symptom intensified while they were smoking and were requested to choose from one of the following options in relation to the frequency of symptom intensification while smoking: 'always', 'often', 'occasionally', 'rarely', and 'never'.

Breath CO measurement

Exhaled CO measurements were performed as reported previously with the Micro-Smokerlyzer (Bedfont Scientific Ltd, UK) sensitive to CO from 0 to 250 p.p.m. by volume. Blood carboxyhemoglobin concentrations were also displayed by the device and recorded in each case.[31]

Tear function parameters

All tear function and ocular surface examinations were carried out under the same settings with the room temperature at 22°C and humidity between 50 and 60%.

Tear evaporation rate
The tear film evaporation rate was measured as described previously by using a tear evaporimeter (KAO Corporation, Tokyo, Japan) following the manufacturer's instructions.[32]

DR-1 lipid layer interferometry
Lipid layer interference images were recorded by the DR-1 interferometer device (DR-1 Kowa Co., Nagoya, Japan) soon after a complete blink.[33] In this study, tear film lipid spread time was defined as the time from the end of a complete blink until a stable lipid layer fringe pattern was attained in the DR-1 interferometer. Lipid spread time was measured three times and the mean value was calculated.

Tear hexanoyl-lysine ELISA analysis
Being a marker for initial stage of lipid peroxidation, we investigated the changes in the tear hexanoyl-lysine (HEL) concentration in response to a possible reactive oxidative species burden. Tear samples were taken from each eye, using a $2 \mu l$ capillary tube to analyse tear HEL concentration. HEL ELISA kits (Nikken Seil Co. Ltd, Shizuoka, Japan) were used to determine the tear HEL concentrations, as reported previously.[34]

Tear film break-up time
Two microlitres of 1% fluorescein and Rose Bengal containing preservative free solution were applied in the conjunctival sac with a micropipette.[35] The standard tear film break-up time (TBUT) measurement was performed by instructing the subjects to blink several times for a few seconds to ensure adequate mixing of the dye. The interval between the last complete blink and the appearance of the first corneal black spot in the stained tear film was measured three times and the mean value of the measurements was calculated. A TBUT value of less than 10 s was considered abnormal.

Ocular vital staining scores
Two microlitres of 1% fluorescein and Rose Bengal containing preservative free solution were applied in the conjunctival sac with a micropipette. Fluorescein staining scores of the corneas ranged between 0 and 9 points. Any score above three points was regarded as abnormal.[35]

Schirmer's I test
For further evaluation of tears, the standard Schirmer's test without topical anaesthesia was performed as

recommended by the 2007 Dry Eye Workshop report.[36] The sterilized strips of filter paper (Showa Yakuhin Kako Co. Ltd, Tokyo, Japan) were placed in the lateral cantus away from the cornea and left in place for 5 min. Readings were repeated in millimetres of wetting for 5 min.[36]

Impression cytology parameters

The impression cytology (IC) specimens were obtained after administration of topical anaesthesia with 0.4% oxybuprocaine. Two separate strips of cellulose acetate filter paper (Millipore HAWP 304, Bedford, MA, USA), that were soaked in distilled water for a few hours and dried at room temperature, were applied on adjacent the medial bulbar conjunctiva, pressed gently by a glass rod, and then removed.

The specimens were then fixed with formaldehyde, stained with Periodic acid-Schiff (PAS), dehydrated in ascending grades of ethanol and then with xylol, and finally mounted with coverslips. Goblet cells were counted in three nonoverlapping areas in each specimen, chosen at random with the help of a calibrated grid. Squamous metaplasia was assessed in the same areas at a magnification of $\times 400$. The goblet cell densities were reported as cells per square millimetre with standard deviations. The specimens were also assigned a grade of conjunctival epithelial squamous metaplasia according to Nelson's grading scheme.[37]

Brush cytology

The brush cytology specimens were obtained after administration of topical anaesthesia with 0.4% oxybuprocaine. An area of medial bulbar conjunctiva, which did not undergo IC, was used for sampling. Conjunctiva was scraped seven times with the Cytobrush-S (Medscand AB, Sweden), the examiner holding the brush 2 cm away from the brush end, applying a gentle pressure to the conjunctiva. After sampling, the brushes were immediately placed in 1 ml of Hank's buffered solution, and the containers were shaken in order to detach the cells from the brush. The suspended cells were collected using the Millipore filter technique employing filters with 8 μm pore size.

The samples were allocated for the assessment of conjunctival inflammatory cell numbers by Diff-Quik staining. The inflammatory cells were counted from 10 nonoverlapping fields and the means were calculated for purposes of this study.

Statistical analysis

Mann–Whitney test was used for statistical comparisons. A *P*-value less than 0.001 was considered statistically significant. Statistical analysis was performed by using Instat (GraphPad software, San Diego, USA).

Results

The cumulative exposure to cigarette smoke was 400 pack years in chronic smokers. None of the control subjects had actively smoked cigarettes and had a history of passive smoke exposure at home or at work in the current study.

Exhaled CO concentrations

Exhaled CO and blood haemoglobin levels were detectable in all subjects. The mean exhaled CO concentration in smokers and control subjects were 16.6 ± 9.7 p.p.m. and 1.3 ± 1.3 p.p.m., respectively (smokers, median: 10 p.p.m., upper 95% CI: 22.07 p.p.m., lower 95% CI: 11.2 p.p.m.; non-smokers, median: 2 p.p.m., upper 95% CI: 2.07 p.p.m., lower 95% CI: 0.58 p.p.m.). The differences were statistically significant ($P < 0.0001$). The mean CO concentration in serum haemoglobin in smokers and control subjects were $4.8 \pm 0.4\%$ and $0.5 \pm 0.5\%$, respectively (smokers, median: 4.8%, upper 95% CI: 5.04%, lower 95% CI: 4.63%; non-smokers, median: 0.9%, upper 95% CI: 0.82%, lower 95% CI: 0.24%). The differences were statistically significant ($P < 0.0001$).

Ocular symptoms

Out of 15 smokers, 12 (80%) reported ocular symptomatology. The worst symptoms included burning sensation, irritation, foreign body sensation, tiredness, and dryness. Eight smokers (53.3%) reported that their worst symptom always intensified after smoking. Three smokers (20%) stated often and one smoker (6.7%) noted occasional intensification of their worst symptom when they smoked. Three smokers (20%) never experienced intensification of their worst symptom after smoking.

Tear function parameters

Tear evaporation rate
The mean tear evaporation rates in smokers and control subjects were 7.7 ± 0.2 and $2.5 \pm 0.9 \times 10^{-7}$ g/cm^2/s, respectively (smokers, median: 7.5, upper 95% CI: 7.87, lower 95% CI: 7.59; non-smokers, median: 2.4, upper 95% CI: 3.03, lower 95% CI: 1.96). The differences were statistically significant ($P < 0.0001$).

DR-1 lipid layer interferometry
The mean lipid spread times in chronic smoker subjects and non-smoker controls were 2.5 ± 0.5. and 1.2 ± 0.2 s,

respectively (smokers, median: 2.34, upper 95% CI: 2.85, lower 95% CI: 2.23; non-smokers, median: 1.17, upper 95% CI: 1.33, lower 95% CI: 1.05). The differences were statistically significant ($P < 0.0001$).

Two types of lipid layer spread abnormality were observed. We observed complete lipid spread after a full blink but with instant appearance of multiple break-up points suggesting tear instability in 22 eyes (Figure 1a). In the other eight eyes, we noted incomplete and irregular lipid spread (Figure 1b). All control subjects had complete spread of the tear film with a complete blink as shown in Figure 1c.

Tear hexanoyl-lysine ELISA analysis
The mean tear HEL levels in smokers and control subjects were 380 ± 18 and 336 ± 20 nmol/l, respectively (smokers, median: 388, upper 95% CI: 390, lower 95% CI: 370; non-smokers, median: 322, upper 95% CI: 347, lower 95% CI: 324). The mean tear HEL value was significantly elevated in smokers compared to the control subjects ($P < 0.0001$).

Tear film break-up time
The mean TBUT was significantly lower in smokers (3.2 ± 0.7 s) compared to non-smoker controls (14.2 ± 2.4 s; $P < 0.0001$; smokers, median: 3, upper 95% CI: 3.57, lower 95% CI: 2.82, non-smokers, median: 14, upper 95% CI: 15.6, lower 95% CI: 12.9).

Ocular vital staining scores
Only one non-smoker control had a positive corneal fluorescein staining (1 point). The mean fluorescein staining score of smokers was 2.86 ± 0.35 points which was significantly higher than the non-smoker controls whose score was 0.07 ± 0.26 points ($P < 0.0001$, smokers, median: 3, upper 95% CI: 3.06, lower 95% CI: 2.67; non-smokers, median: 0, upper 95% CI: 0.22, lower 95% CI: 0.08).

Schirmer's test
There was no significant difference in Schirmer's test values between smokers (13.3 ± 2.1 mm) and non-smoker controls (17.1 ± 2.6 mm; $P > 0.05$).

Impression cytology parameters
The mean squamous metaplasia grade was significantly higher in smokers than the controls ($P < 0.0001$). The mean goblet cell density was significantly reduced in smokers compared to non-smoker controls as shown in Figure 2a ($P < 0.0001$). IC specimens from healthy control subjects revealed numerous goblet cells, abundant mucin pickup, and sheets of normal conjunctival epithelial cells (Figure 2b). Specimens from smokers revealed either a few or no goblet cells and epithelial cells with advanced squamous metaplasia (Figure 2c–d).

Brush cytology for inflammatory cell counts
BC revealed decreased intercellular cohesiveness and a significantly higher number of neutrophils in smokers compared to non-smoker controls (Figure 3a). BC specimens from control subjects did not reveal any inflammatory cells (Figure 3b). BC specimens from smokers showed numerous neutrophils, some interspersed among sheets of conjunctival epithelial cells (Figure 4).

Discussion

Cigarette smoking has been reported to be one of the factors in the array of clinical conditions, resulting in a dysfunctional tear film.[38,39] It is well known that the tar and gas phases of tobacco contain many oxidizing substances exposing inhalers to an enormous free radical load.[40] Although the ocular surface is the most environmentally exposed mucosal surface of the body, including tobacco smoke exposure, only four studies in the literature reported data concerning the effects of
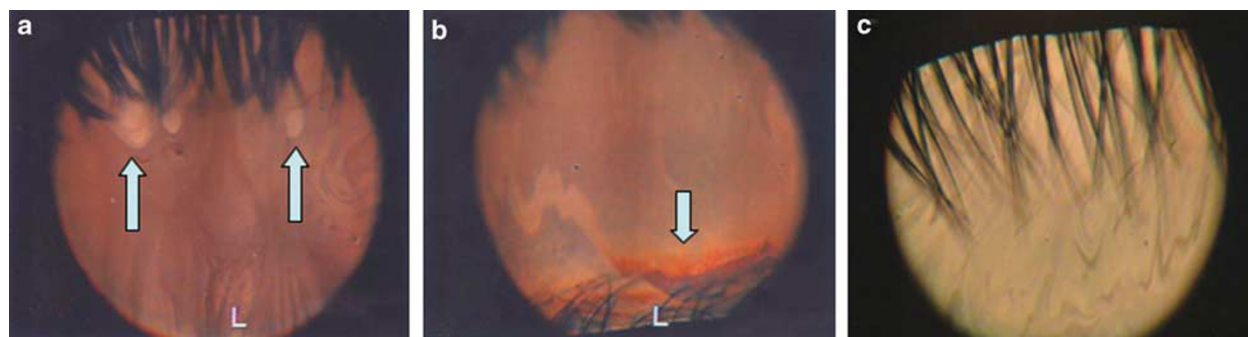

Figure 1 DR-1 tear film lipid layer observations in chronic smokers. (a) Type I abnormality: note the complete spread of the tear film lipid with multiple lipid layer break up points (blue arrows) soon after the full blink. (b) Type II abnormality: note the incomplete and irregular spread of the tear film lipid layer soon after the full blink (blue arrow). (c) Normal tear film: note the complete spread of the tear film lipid layer with a full blink in a normal subject.
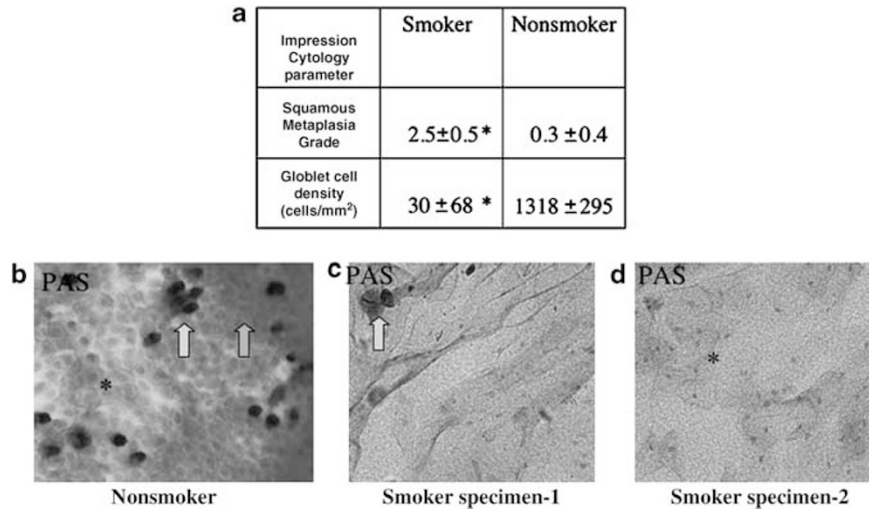
a Impression Cytology parameter	Smoker	Nonsmoker
Squamous Metaplasia Grade	2.5±0.5*	0.3 ±0.4
Globlet cell density (cells/mm²)	30 ±68 *	1318 ±295

Figure 2 Comparison of impression cytology findings between chronic heavy smokers and non-smoker controls. (a) Note the significantly higher squamous metaplasia grade and the significant reduction in goblet cell density in chronic heavy smokers. (b) Note the presence of numerous goblet cells (blue arrow), sheets of healthy conjunctival epithelial cells (asterisk mark) and abundant mucin pickup by the filter paper (pink arrow). (c) Note the dramatic reduction of goblet cell numbers (blue arrow). (d) Note the advanced squamous metaplasia (asterisk mark) and absence of goblet cells in another representative specimen.
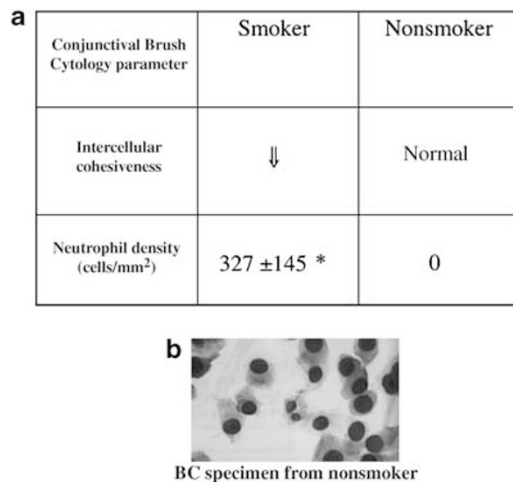
a Conjunctival Brush Cytology parameter	Smoker	Nonsmoker
Intercellular cohesiveness	⇓	Normal
Neutrophil density (cells/mm²)	327 ±145 *	0

BC specimen from nonsmoker

Figure 3 Comparison of brush cytology findings between chronic heavy smokers and non-smoker controls. (a) Note the lower neutrophil density in the heavy smokers. (b) Note the healthy conjunctival epithelial cells and absence of any neutrophils in the specimen from a non-smoker control subject.

cigarette smoke on the ocular surface and tear film characteristics.[38,39,41,42] All four studies focused on the effects of active chronic smoking and concluded that smoking was associated with tear instability and deteriorating effects on the ocular surface health. Chronic smokers were also reported to have low corneal and conjunctival sensitivity,[39] increased conjunctival squamous metaplasia,[39] and alteration of tear proteins.[7,41,42] In our previous study, we found tear film instability and abnormal lipid layer interferometry patterns in chronic smokers attributing these changes to a

possible tear film lipid layer damage.[39] In accordance with studies on tear film stability changes in chronic smokers, we reconfirmed significant perturbation of the tear film stability in our current work. We report a significant increase in tear evaporation rate and slowing of the tear film lipid spread time in chronic smokers compared to non-smoker controls for the first time in the literature. It is believed that the tear film lipid layer minimizes the evaporation of the aqueous component of the tear film in physiologic states and in adverse environments.[43] The lipids, aqueous portion, and mucin components of the tear film are believed to interact with each other to achieve an even spread of the lipid layer in the tear film, rendering the cornea wettable and allowing tear stability within the normal limits.[43] As none of the smoker subjects had an ocular/eyelid disease including blepharitis, infectious keratoconjunctivitis, and meibomian gland dysfunction in this study, the prolongation of lipid spread time consistently in all of our chronic smoker subjects may be explained by possible mucin changes in the ocular surface as evidenced by a reduction of goblet cell density and possible changes in the nature of tear lipids, as evidenced by increased tear HEL levels. Lipids are known to be potential targets for oxidative attack of free radicals. HEL is a useful biomarker for initial stage of lipid peroxidation, which can be detected in human atherosclerotic lesions, urine, serum, and tears.[44] It should be noted though that the increase in tear HEL levels might not necessarily be a toxic change resulting from lipid peroxidation, but may be a physiological response to an increased reactive oxygen species burden.
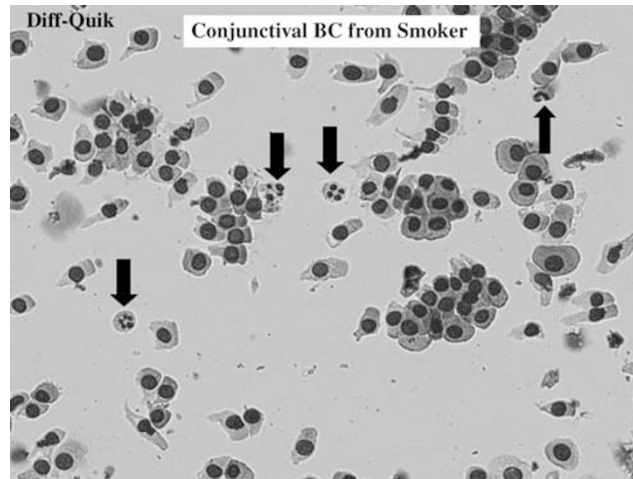
Figure 4 Representative conjunctival brush cytology specimen from a chronic heavy smoker subject. Note the presence of numerous neutrophils (blue arrows), which was a consistent finding from specimens of smoker subjects.

Increased tear hexanoyl-lysine in chronic smokers might have induced functional changes in the tear lipids and might explain the lipid spread alterations observed in the current study. Another explanation may be the decrease in conjunctival goblet cells in smokers which solely secrete MUC5AC into the tears, the major mucin thought to be responsible for the attainment of tear stability.[45] A concomitant reduction in MUC5AC quantity in tears might have altered the interaction of mucins with tear lipids which should be investigated in future trials. A previous study did not reveal significant differences in goblet cell density between smokers and non-smoker controls, a finding which was different from the current study.[38] The smoker subjects of this study were all symptomatic long-term smokers with a smoking history of greater than 20 years where the subjects of the previous report included chronic smokers with a variable duration of smoke exposure which may explain these differences.

BC examination also provided interesting findings. All BC specimens from the smokers in the current study consistently revealed neutrophil infiltrates, the densities of which were significantly higher compared to the non-smoker controls. Although not investigated in the current study, an increase in ocular surface and tear inflammation[46] has been shown to coexist with increased levels and activity of proteolytic enzymes, including plasmin and matrix metalloproteases, in dry eye syndromes resulting in ocular surface epithelial damage[47–50] which might explain the higher fluorescein scores observed in the smokers.

In summary, our study confirmed the deleterious effect of chronic cumulative tobacco smoke exposure on the tear film and ocular surface health status. It would be interesting to investigate the effect of passive exposure to cigarette smoke on the ocular surface, tear film and visual status, an issue still uninvestigated in ophthalmic literature. It is our sincere wish that the adverse findings following chronic long-term cigarette-smoke exposure observed in this study alert all professionals for adoption of prevention policies especially by public and health officials.

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