CLINICAL STUDY

The role of tear physiology in ocular surface temperature

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Abstract

Purpose **To determine whether the more rapid cooling of the tear film in dry eyes is related to other tear film parameters, a battery of tear physiology tests was performed on dry eye patients and control subjects.**
Methods **Tear evaporation rate was measured with a modified Servomed (vapour pressure) evaporimeter and ocular temperature with an NEC San-ei 6T62 Thermo Tracer in 9 patients diagnosed as having dry eye and in 13 healthy control subjects. Variability in temperature across the ocular surface was described by the temperature variation factor (TVF). Lipid layer structure and tear film stability were assessed with the Keeler Tearscope and tear osmolality was measured by freezing point depression nanolitre osmometry.**
Results **The data were explored by principal component analysis. The subjects with and without dry eye could be separated into two distinct groups entirely on the basis of their tear physiology. Dry eye patients exhibited higher tear evaporation rates, osmolalities and TVF, lower tear film stabilities and poorer-quality lipid layers than the control subjects. A significant linear relationship was found to exist between tear evaporation rate and TVF for all subjects ($R^2 = 0.242$, $p = 0.024$).**
Conclusions **Rapid cooling of the tear film in dry eyes appears to be related to the reduced stability of the tears and the increased rate of evaporation. The higher latent heat of vaporisation, associated with the increased evaporation in dry eyes, may account for the increased rate of cooling of the tear film in this condition.**

Key words Dry eye, Evaporation, Physiology, Tear film, Temperature, Thermography

Non-invasive ocular thermographic assessment was introduced in 1968 by Mapstone and allowed evaluation of normal and pathological conditions.[1–4] Ocular surface temperature in his work was measured with a thermistor, the resistance of which changed with exposure to infrared radiation. New generation thermographic instrumentation has improved the accuracy with which ocular temperatures can be assessed in healthy, pathological, and contact lens wearing eyes.[5–7]

The role of the tear film in surface temperature received little consideration in the early literature. More recently Hädrich[8] categorised the formation of the tear film by the change in temperature and related it to tear film stability. A comprehensive study examining normal and pathological eyes, reported by Morgan *et al.* in 1995,[9] noted a variation in temperature across the normal corneal surface; specifically, that the cornea is warmest at the limbus and coolest over the centre. In dry eyes, it was noted that the mean ocular surface temperature was higher than for normal eyes, which was attributed to the conjunctival hyperaemia associated with this condition. It was also shown that the rate of cooling of the tear film over the centre of the cornea was much faster and consequently the temperature differential between the limbus and the corneal centre was higher in dry eyes than in normal eyes[9] (Fig. 1). The increased rate of cooling may be due, in part, to the larger difference in temperature between the eye and the atmosphere (since the dry eye is initially warmer than the normal eye), but it was suggested that it might also be related to the rate of evaporation of the tear film.[10] When a substance changes its state, heat is transferred to or from the surroundings.[11] As the tear film evaporates, the ocular surface potentially cools due to the positive latent heat of vaporisation as the liquid changes into gas and heat is transferred to the atmosphere.

The evaporation rate of tears has been shown by various workers[12,13] to increase in pathological dry eye. Increased tear evaporation has been found to be related to poor lipid layer quantity or quality, both in the rabbit[14,15] and in the human eye.[16] This increased evaporation rate may be responsible, at least in part, for the increased rate of cooling of the tear film observed in dry eyes. To investigate this hypothesis, a study was conducted in which ocular surface temperature, tear film evaporation and lipid layer integrity were compared in a group of dry eye patients and controls subjects.

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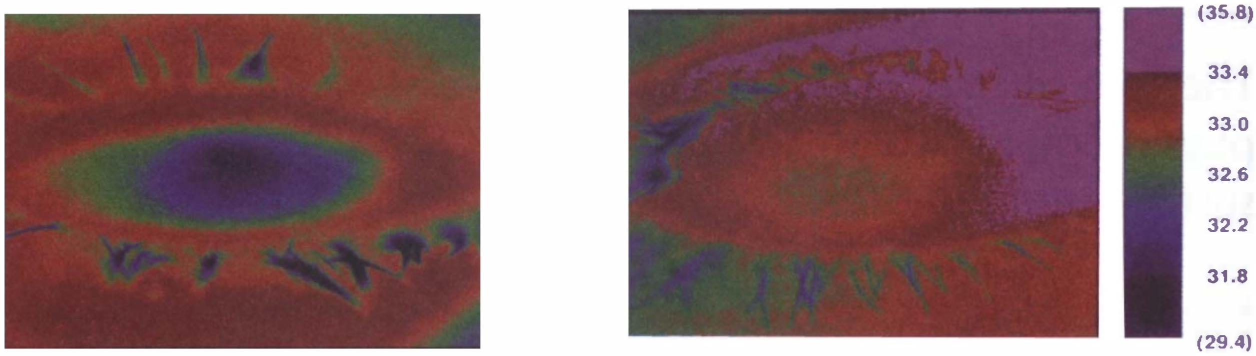

Fig. 1. *Thermograms for a dry eye and a normal eye are compared on the same temperature scale. The dry eye on the left demonstrates a larger difference in temperature between the limbus and the central cornea (approx. 2 °C) than does the normal eye on the right (approx. 0.5 °C).*

Methods

Subjects

Thirteen healthy volunteers (7 men, 6 women) with a mean age ± SD of 24.8 ± 4.1 years (range 19–32 years) participated in the study. The control subjects were asymptomatic of dry eye, had no known ocular or general pathology and were not taking topical or systemic medication. Eight individuals previously diagnosed as dry eye patients by ophthalmologists at Manchester Royal Infirmary, on the basis of symptoms, fluorescein break-up time, Schirmer test and/or rose bengal staining (4 men, 4 women), with a mean age (± SD) of 60.3 (± 18.1) years and an age range of 22–85 years, were recruited for the study. Dry eye was confirmed on the day of assessment by a non-invasive break-up time (NIBUT) of less than 20 s[17] and two or more symptoms of dry eye according to the McMonnies Dry Eye questionnaire.[18] Interferometric assessment identified the tear films of 7 of the 8 dry eye patients as lipid-deficient. The only medication taken by some of the dry eye patients was an artificial tear supplement.

Conditions

The research followed the tenets of the Declaration of Helsinki and informed consent was obtained from each of the volunteers prior to the study. Patients were requested to refrain from instilling any drops and from wearing eye make-up on the day of examination. The measurements for each subject were made consecutively within a 30 min period at Manchester Royal Eye Hospital. The mean temperature and relative humidity (± SD) throughout data collection were 23.1 ± 2.3 °C and 47.1 ± 7.7%, respectively. Measurements were made only on the left eye and in the same sequence for each subject. Tear evaporation rate was assessed first, followed by assessment of the lipid layer and tear film stability. Samples for evaluation of tear osmolality were then collected and stored for analysis at a laboratory remote from the site of collection. A period of 10 min elapsed between osmolality sample collection and thermographic assessment to allow any reflex tearing to subside.

Techniques

Tear evaporation rate was measured with a modified Servomed Evaporimeter (model EP1) as described previously.[16] Two vapour pressure sensors, located within a modified swimming goggle, at a known distance from the evaporating ocular surface recorded the difference in vapour pressure between the sensors over a 30 s period and, from this, derived the rate of evaporation from the surface. These data, which were collected when the eyes were both open and closed, were stored by computer before analysis. The closed eye evaporation results were used to factor out the effect of evaporation from the facial skin within the goggle. Final tear evaporation values were computed from the stored data, the ambient environmental conditions and the area of the eye, which was obtained photographically.

Tear lipid layer structure and NIBUT were assessed with the Keeler Tearscope. This diffuse cold light source was used in conjunction with a non-illuminated biomicroscope to view the lipid layer of the tears interferometrically. Patterns observed were classified, according to the work of Guillon *et al.*, into seven categories on the basis of their appearance,[17] and, from these, lipid layer thickness could be estimated.[16] Tear film stability (NIBUT) was also measured non-invasively with the Keeler Tearscope as the time which elapsed between a blink and the observation of a discontinuity of the lipid layer. Five NIBUT measurements were made in each case and the mean calculated.

Osmolality was determined by freezing-point depression nanolitre osmometry as described in the literature.[19–21] A 0.2–0.4 μl tear sample was collected from the inferior tear meniscus with a salt-free glass microcapillary which had been drawn to a fine point across a flame, and stored beneath cooled, high-viscosity oil prior to analysis. All samples for osmometry were analysed within 1 week of collection. The Clifton Technical Physics Nanoliter Osmometer was employed to evaluate the tonicity of the tear samples relative to standard solutions of purified water and 290 mosmol/kg sodium chloride, as described in earlier work.[19]

Table 1. *Means and standard deviations of the values for tear physiology measurements and ocular surface temperature*

Summary	Controls	Dry eye
Number	13	8
Gender distribution	7M, 6F	4M, 4F
Age (years)	24.8 ± 4.1	60.3 ± 18.1
Evaporation rate ($g/m^2/h$)	0.07 ± 0.51	1.48 ± 0.70
Osmolality (mosmol/kg)	302.9 ± 4.1	314.9 ± 6.7
NIBUT (seconds)	24.1 ± 16.7	6.1 ± 5.1
Lipid layer (modal pattern)[a]	F	CF(ab)
Central corneal temperature (°C)	33.82 ± 0.36	33.24 ± 0.78
TVF (°C)	0.17 ± 0.73	0.24 ± 0.06

NIBUT, non-invasive break-up time; TVF, temperature variation factor.
[a]As defined by Guillon and Guillon.[36]

Ocular temperature measurements were made with an NEC San-ei 6T62 Thermo Tracer, which comprised a detector unit, a control unit/monitor and a thermocorder.[9] Infrared radiation in the region 8–13 μm, incident upon the detector, resulted in an increase in conductivity. This produced a current proportional to the difference in temperature between the object of interest and an object of known temperature. The direct current leaving the detector unit was received and interpreted by the control unit, and a colour-coded image displayed on the monitor. A 'close-up' lens was used in addition to the available zoom facility on the instrument to allow adequate visualisation of the ocular area. Connection of the Thermo Tracer processor unit to an IBM-compatible microcomputer enabled the images to be stored and manipulated by appropriate software. Digital information was processed by NIH Image 1.55 software package.

Since the temperature measurement was more sensitive to air circulation than other tests, the thermographic assessment took place in a cubicle which was formed from non-reflective blackout curtains. In each case the subject was required to have been awake for at least 2 h and to undergo a 20 min adaptation period to the environmental conditions within the cubicle prior to examination. The temperature and relative humidity within the cubicle were monitored by a thermo-hygrometer. The subject's head was supported by a rest for the forehead and chin in front of the instrument. To prevent air currents arising from movements of the examiner, alignment of the instrument was performed remotely by means of a joystick connected to the detector. Recording of the thermogram began 4–5 s after a 3 s period of eye closure in each case to minimise any effect on temperature measurements caused by the inflow of fresh tears with each blink.

Eight thermograms were recorded for the left eye of each subject. Central corneal temperature (CCT) was determined by recording the mean of the pixels, representing temperature values, across the cornea. The variation in temperature across the cornea was represented by the standard deviation of the pixel temperature values, termed the temperature variation factor (TVF).

The data from this study were derived from a large number of inter-related variables. To reduce the dimensionality of the data set while retaining as much of the variation present in the original data as possible, principal component analysis was carried out.[22]

Results

Mean values (± SD) for the measured parameters are summarised in Table 1. The principal component analysis[23] applied in the initial assessment of the data showed that the first two principal components (PC 1 and PC 2) explained 65% of the original variation in the data. The coefficients for PC 1 and PC 2 are shown in Table 2. These coefficients can be used to calculate principal component scores for each of the subjects using equations (1) and (2):

$$\text{PC 1} = 0.06 \times \text{TEMP} - 0.11 \times \text{HUM} - 0.23 \times \text{NIBUT} + 0.30 \times \text{OSM} + 0.30 \times \text{EVAP} - 0.22 \times \text{CCT} + 0.30 \times \text{TVF} \quad (1)$$

$$\text{PC 2} = -0.48 \times \text{TEMP} + 0.47 \times \text{HUM} - 0.07 \times \text{NIBUT} + 0.07 \times \text{OSM} + 0.19 \times \text{EVAP} + 0.02 \times \text{CCT} + 0.03 \times \text{TVF} \quad (2)$$

Fig. 2 shows a plot of the scores for PC 2 against those for PC 1. From this figure, it can be seen that the control subjects exhibited low PC 1 values in comparison with the dry eye patients, highlighting significant basic

Table 2. *Coefficients for each of the variables for the first two principal components, PC 1 and PC 2. The table also shows abbreviations used in equations (1) and (2)*

Variable	Abbreviation	Coefficients PC 1	Coefficients PC 2
Room temperature (°C)	TEMP	0.06391	−0.47857
Room relative humidity (%)	HUM	−0.11195	0.46873
NIBUT (seconds)	NIBUT	−0.23277	−0.07207
Osmolality (mosmol/kg)	OSM	0.29992	0.07859
Evaporation rate ($g/m^2/h$)	EVAP	0.30034	0.19082
Central corneal temperature (°C)	CCT	−0.22265	0.02090
TVF (°C)	TVF	0.30334	−0.03276

NIBUT, non-invasive break-up time; TVF, temperature variation factor.

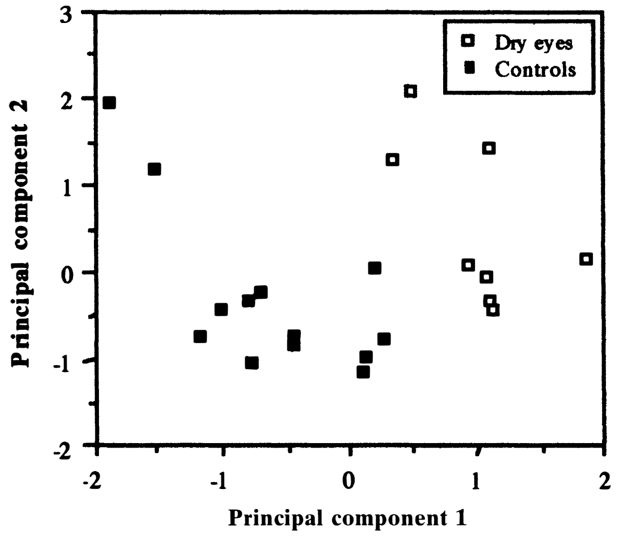

Fig. 2. *Data were explored by principal component analysis. The scores for the first principal component are plotted against the scores for the second. Filled markers, controls, hollow markers, dry eye patients.*

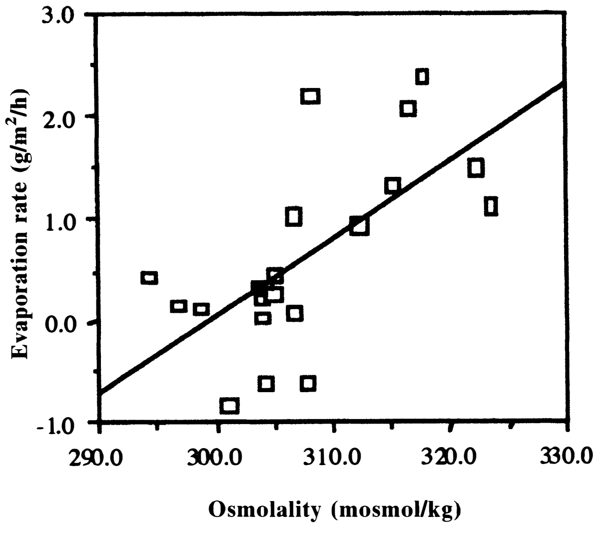

Fig. 3. *A linear relationship was demonstrated between tear osmolality and evaporation rate (*R^2 *= 0.428, p = 0.001) for all subjects. The regression line is included on the graph.*

differences between the groups of subjects, as described below. Table 2 shows the coefficients attached to the original variables for each of the two components. The variables with negative coefficients act in opposition to those with positive coefficients. In PC 1, the variables having most effect are the parameters describing tear physiology: NIBUT, evaporation, osmolality, central corneal temperature and TVF. A high (or positive) value for PC 1 (as established for the dry eye patients) would require a low stability, high evaporation rate, high osmolality, low central corneal temperature and high TVF – factors associated with poor tear physiology. In contrast, the controls, who exhibited low PC 1 values, exhibited high tear film stability and central corneal temperature together with low tear evaporation rate, osmolality and TVF.

PC 2 appeared to separate the subjects primarily on the basis of the environmental conditions on the day of examination. There were no obvious groupings for the subjects with either a high or a low score for PC 2. No significant difference was found between the room temperatures or humidities to which the control subjects or dry eye patients had been exposed (Student's *t*-test, $p = 0.260$ and $p = 0.423$ respectively).

In previous studies[9,16,19] all measured parameters have been found to be normally distributed except for lipid layer structure and tear film stability. Natural log transformation of the positively skewed NIBUT distribution rendered these data suitable for parametric statistical analysis. Thus, to compare the tear physiology of the dry eye patients with that of the control subjects, a Student's *t*-test was performed on the variables describing tear physiology. Mean tear evaporation rate and osmolality were found to be significantly higher in the dry eye patients than in the control subjects ($p < 0.001$ in both cases). For the transformed means of NIBUT, it was found that the tear film stability of the controls was significantly higher than that of the dry eye patients ($p < 0.001$). Similarly, mean central corneal temperature was found to be significantly higher in the control group than in the dry eye group ($p = 0.032$). There was a statistically significant difference in the variation of temperature across the ocular surface such that TVF was found to be higher for the dry eye group than for the controls ($p = 0.024$). There were no gender-related differences in the results ($p > 0.05$ in each case).

Significant inverse linear relationships were demonstrated between the transformed values of tear film stability and both tear evaporation rate ($R^2 = 0.325$, $p = 0.007$) and osmolality ($R^2 = 0.251$, $p = 0.021$) for all subjects. Tear evaporation rate was found to be linearly related both to tear osmolality ($R^2 = 0.428$, $p = 0.001$), as shown in Fig. 3, and to TVF ($R^2 = 0.242$, $p = 0.024$), as illustrated in Fig. 4. TVF was also found to be inversely related to the central corneal temperature ($R^2 = 0.233$, $p = 0.027$).

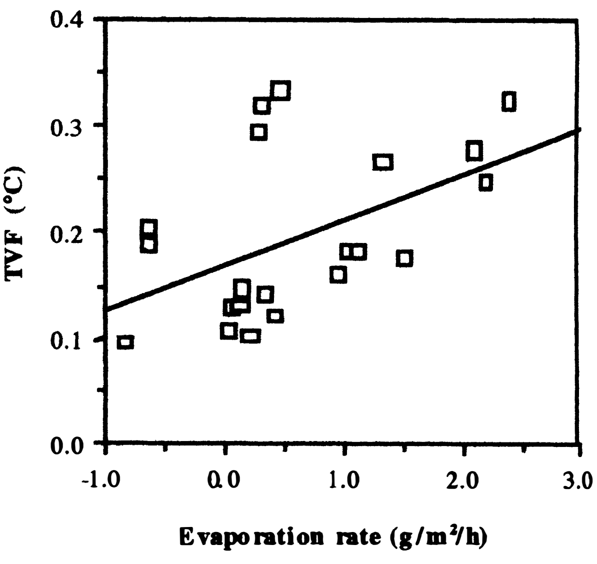

Fig. 4. *A linear relationship between temperature variation factor (TVF) and tear evaporation rate (*R^2 *= 0.242, p = 0.024) was found to exist. The regression line is shown on the scatter plot.*

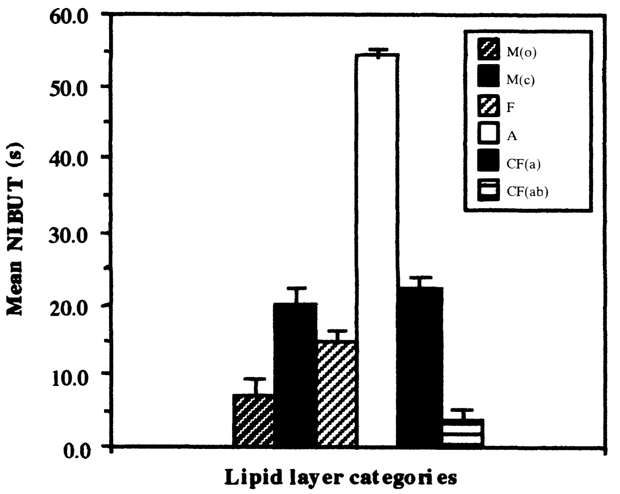

Fig. 5. *The graph shows mean non-invasive break-up time (NIBUT) values for the lipid layer categories as defined by Guillon and Guillon.[36] M(o), open meshwork marmoreal; M(c), closed meshwork marmoreal; F, flow; A, amorphous; CF(n), normal-coloured fringe; CF(a), abnormal-coloured fringe. In the ANOVA of these data, the natural log transformation was employed in view of the positively skewed distribution of raw data.*

To determine the effect of the lipid layer on the measured aspects of tear physiology, a one-factor analysis of variance (ANOVA) was performed. The type of lipid layer pattern exhibited by an individual was found to have a significant influence on both the tear film stability ($p = 0.002$) and osmolality ($p = 0.010$). A bar chart of the mean tear film stability for each of the lipid layer categories is shown in Fig. 5. Tear film stability was observed to be significantly lower in individuals with an open meshwork marmoreal pattern compared with those with a closed meshwork marmoreal, an amorphous or a normal-coloured fringe pattern (Fisher PLSD post-hoc test, $p < 0.05$). Subjects with an abnormal-coloured fringe lipid pattern were also found to exhibit significantly lower tear film stabilities than all groups except those with an open meshwork pattern. A significantly lower tear film stability was found in those exhibiting a flow lipid pattern than in those with an amorphous pattern. In the case of tear osmolality, the only significant difference was found for those with an abnormal-coloured fringe pattern compared with those exhibiting other lipid layer patterns. This group with an abnormal-coloured fringe was found to have a particularly high tear osmolality (ranging from 315.1 to 323.5 mosmol/kg). The lipid layer of the tears was shown, by ANOVA, not to affect the evaporation rate ($p = 0.311$), ocular temperature ($p = 0.988$) or TVF ($p = 0.349$).

Discussion

Ideally, the two groups of study subjects would be age-matched to control for any effect of age on the tear parameters. Unfortunately this was not possible within the time constraints of the data collection, introducing a potential bias to the results. However, no effect of age on tear evaporation has been demonstrated in the literature.[24,25] Recent work showed that there was a significant effect of age on tear osmolality for females ($R^2 = 0.164$, $p = 0.006$), but when both males and females were considered together there was no significant age effect ($p > 0.05$).[19] Similarly, when gender is accounted for, in a study by Mathers *et al.*,[26] no significant age effect is observed.[27] There are varying opinions in the literature regarding a relationship between tear film stability and age.[28–31] However, no significant relationship between tear film stability and age has been established by the authors in earlier work.[25] Thus, although age-matching of the controls would have been preferable, the absence of conclusive relationships between age and tear film stability, evaporation and osmolality, suggests that lack of control for age in the current investigation may not be crucial.

Mean ocular surface temperature has been shown to decrease with age, but an exact rate has not been agreed upon.[32–34] With the instrument used in the current study, the rate of decrease of temperature with age, attributable to vascular constriction, was found to be –0.008 °C per year.[9] A correction factor to account for the decrease in ocular temperature with age was not used, however, due to the relatively complex nature of this relationship[9] and the compounding fact that age cannot be regarded as being independent of ocular or general disease processes. Moreover, the decrease in ocular surface temperature has been demonstrated only for the mean ocular surface temperature and not for the central corneal temperature, which was measured in this study.

For the control group the mean tear evaporation rate (± SD) was 0.07 ± 0.51 g/m^2/h. This falls within the normal range of evaporation rates established for the instrument used in this study.[14] The mean tear evaporation rate of the dry eye group was 1.48 ± 0.70 g/m^2/h. Although this mean would appear to fall within the upper range of normal evaporation rates, it was significantly higher than that of the controls. One research group in Japan has observed decreased tear evaporation in dry eye;[35] however, most investigators, in agreement with the findings of this study, have shown significantly higher rates of tear evaporation in dry eye compared with normal eyes.[12,13]

The mean tear osmolality (± SD) of 302.9 ± 4.1 mosmol/kg for the control group compares well with published values.[20,21] Although the mean tear osmolality (± SD) observed for the dry eye group (314.9 ± 6.7 mosmol/kg) falls outside the range of normality defined by Gilbard *et al.*,[20] it falls within the limits (312–323 mosmol) defined only as 'suspect dry eye' in previous work. The reason for this is unknown but may reflect the small sample size. The mean osmolality value (± SD) for the dry eye group was, however, significantly higher than that for the control group.

NIBUT measurements appear to be in agreement with published values for the Tearscope.[36] Reduced values of tear film stability in dry eye conditions have been reported by various authors[37–39] and were also observed in the current study. The lipid layers were divided into six categories based on the pattern visible with the

Tearscope.[17] The most common pattern in the control group was the flow pattern. This is acknowledged to be a fairly stable pattern and has been reported to be the most frequently observed lipid layer pattern in normal eyes.[36] In the dry eye group, the most commonly observed pattern was the abnormal-coloured fringe pattern. This features clumps of lipid floating amidst areas of exposed aqueous. It is most often exhibited by patients with dry eye[36,40] and is associated with poor tear film stability.

The dry eye patients were found to have a significantly lower central corneal temperature than the controls in this study. In the study by Morgan *et al.*[9] there was no significant central corneal temperature difference between the control subjects and the dry eye patients, although the dry eye patients had a significantly higher mean ocular surface temperature than did the normals. The mean ocular surface temperature was not quantified in this study since the central corneal temperature and the temperature variation factor were considered superior in the assessment of dry eyes. The research group in Japan who have also studied central corneal temperature in patients with dry eye[41,42] found it to be higher in dry eye patients than in controls. They, too, believe that evaporation rate and tear film temperature are related but explain their results in terms of the reduced evaporation which they demonstrate in dry eye.[35] The reason for the conflicting results is unknown, but may reflect the different populations of dry eye patients in the respective studies. In the current study, many patients exhibited poor lipid and increased tear evaporation, indicating an 'evaporative' dry eye.[43] In contrast, and as acknowledged by the authors, the subjects in the Japanese study appear to have comprised primarily 'aqueous-deficient' dry eye patients, possibly accounting for the differences.[42] In these particular studies, tear evaporation rates and tear film temperatures were not measured at the same visit, preventing direct correlation of the parameters in these eyes.[41,42]

The primary source of ocular radiation measured by thermography has been shown to be the tear film.[9] Beneath the closed lids, the temperature of the central cornea would be expected to be the same as that of the palpebral conjunctiva. However, when the lids are open, the ocular surface loses heat by convection, radiation and evaporation, and the centre of the cornea becomes cooler than the limbus. The avascular nature of the cornea precludes expeditious heat transfer from the anterior ocular surface vasculature to the centre of the cornea and hence, following a blink, this area cools most rapidly. An additional, smaller effect may arise from varying evaporation of the tear film across the surface. Absence or poor confluence of the lipid layer results in a large increase in the rate of evaporation.[16] Therefore, areas of incomplete lipid coverage (and higher evaporation) following tear break-up after a blink may be expected to show greater heat loss. This could include areas of central cornea.

In dry eyes, the temperature differential across the cornea was found to be greater as evidenced by the larger TVF. This indicated that the change in temperature over the surface was more variable in dry eyes, due to localised areas of cooling and possibly to a greater temperature differential between the limbus and the central cornea.

Morgan *et al.*[9] found the mean ocular surface temperature of the dry eye to be greater than that of the normal eye and attributed this to the higher level of vascular activity in the limbal region. However, when sequential thermograms separated by 1 s were compared for dry eyes and controls, it was found that the *rate* of cooling was much faster in dry eyes.[10] In the current study, the mean ocular surface temperature was not evaluated, but it was found that the central corneal temperature was significantly lower in the dry eye patients than in the control subjects. TVF was found to be inversely related to central corneal temperature; thus the subjects with the higher variation in temperature across the ocular surface tended to have lower corneal temperatures.

It is possible that the rapid decrease in tear film temperature in dry eyes is a result of increased evaporation rate. If, on eye opening, the evaporation rate of the tear film of the dry eye is higher than that of the control eye, within several seconds the cooling of the cornea of the dry eye will be greater than that of the control eye due to the greater effect of the positive latent heat of vaporisation of its tear film. It was found in the current experiment that the dry eye patients did have a significantly higher tear evaporation rate than the controls (Table 1). In fact, a significant linear relationship was found to exist between the rate of evaporation and the TVF for the group of 21 dry eye patients and control subjects in this study (Fig. 4), supporting the hypothesis that the cooling of the central cornea is related to the evaporation of the overlying tear fluid.

Not unexpectedly, tear evaporation rate and osmolality were found to be positively related as shown in Fig. 3, and both tear evaporation rate and osmolality were found to be inversely related to stability – results which are logical and consistent with previous observations.[44]

The stability of the tear film was found to depend significantly upon the lipid layer pattern observed with the Tearscope. A poor lipid layer (particularly an abnormal-coloured fringe pattern) could be a contributory cause of increased cooling of the central cornea as a result of its inability to retard evaporation.[16] In a tear film with a compromised lipid layer it would seem logical that the lipid cover over the central area of the cornea is poorest, since not only is this area the furthest from the lids which spread the available lipid by blinking, but it is also the site of the greatest change in curvature of the ocular surface. This is supported by the clinical impression gained from use of the Tearscope in such eyes, where tear break-up is apparent, almost immediately after a blink, near the centre of the cornea rather than peripherally. The lack of relationship between NIBUT and evaporation rate in the current work may be due to the evaporation measurement taking into account the whole of the exposed ocular surface, and not just the central corneal area observed with the Tearscope.

Conclusion

The dry eye patients and control subjects in this study could be separated into two distinct groups on the basis of their tear physiology. Dry eye patients exhibited a lower tear film stability and central corneal temperature in conjunction with higher tear film evaporation, osmolality and temperature variation across the ocular surface. The study has further demonstrated that the tear film is an important determinant of ocular surface temperature in normal and dry eyes. An unstable tear film with high evaporation rate and resultant increased osmolality contributes to the characteristic temperature profile of the dry eye.

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