Effect of Tear Hyperosmolarity and Signs of Clinical Ocular Surface Pathology upon Conjunctival Goblet Cell Function in the Human Ocular Surface

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PURPOSE. To investigate the effect of tear hyperosmolarity and signs of clinical ocular surface pathology on conjunctival goblet cell population.

METHODS. 111 participants were assessed using tear osmolarity (TO) measurements and a comprehensive selection of clinical ophthalmic tests. The resultant clinical database was assessed for evidence of patterns of composite increasing pathology. The total, filled, and empty goblet cell numbers were measured: total number of goblet cells as per cytokeratin 7 (CK7) immunofluorescence and number of filled goblet cells as per periodic acid Schiff’s reagent (PAS) or lectin helix pomatia agglutinin (HPA). Goblet cell profile was correlated with composite clinical pathologic grades.

RESULTS. No significant correlation was found between TO and goblet cell number or function (as indicated by number of filled or unfilled goblet cells). Distinct composite clinical pathologic groups 0–IV with increasing pathology were created based on the frequency of positive pathologic signs, which adhered to the Dry Eye Workshop purported mechanism. Only in group IV was there significantly increased mean tear osmolarity of 344 mOsm/L (P < 0.000) along with significantly decreased empty goblet cell number (CK7- and HPA-) compared to filled (CK7+ and HPA+, P = 0.000). When the number of filled goblet cells (PAS+) was analyzed there was significant increase in tear osmolarity for the most two severe grades; 3 and 4.

CONCLUSIONS. The goblet cell population does not appear to be affected by isolated tear hyperosmolarity. Hyperosmolarity when combined with other ocular surface pathology or inflammation alters the goblet cell population. (Invest Ophthalmol Vis Sci. 2011;52:6174–6180) DOI:10.1167/iovs.10-7022

Dry eye is characterized in the Dewes report1 to be associated with tear hyperosmolarity and instability, resulting in a number of symptoms, including dry, gritty, burning, or even watery eyes.2,3 Osmolarity has been deemed to be the key diagnostic test for dry eye for many years and proposed as a gold standard test for dry eye in the First International Conference on the Lacrimal Gland, Tear Film and Dry Eye in 1992.4

Hyperosmolarity is recognized to be an important mechanism within the spectrum of dry eye disease; however, its relationship to the spectrum of signs and symptoms characteristic of dry eye disease remains to be fully elucidated.1 It has been reported, however, as potentially the key mechanism for induction of the entire spectrum of ocular surface pathology produced within dry eye syndrome, principally through inducing inflammation, cell death, and destabilizing the tear film.1 Much debate continues regarding whether hyperosmolarity is a consequence or cause of the overt clinical signs and symptoms of dry eye.

Tear hyperosmolarity arises as a result of water evaporation from the exposed ocular surface in situations of low aqueous tear flow, excessive evaporation, or a combination of these events. Tear osmolarity is believed to be higher in the tear film itself than in neighboring menisci, perhaps because the ratio of area to volume is higher in the tear film than the menisci.1 Low lacrimal flow can cause tear hyperosmolarity, which indirectly through activation of inflammatory pathways may affect the density of filled (periodic acid, alcian blue/Schiff’s reagent, PAS+) goblet cells5 and/or goblet cell secretion. Changes in goblet cell secretion may in turn contribute to tear film instability and directly alter tear osmolarity. Inflammation, however, can also be present on the ocular surface due to causes other than dry eye. This potentially can further contribute to deleteriously affect the functioning goblet cell, resulting in eventual tear film destabilization. Within this study subjects were characterized using evidence of increasing levels of clinical inflammation based on an initial tear osmolarity reading of ≥308 mOsmol/L along with clinical slit lamp patterns of composite inflammation and other significant ocular surface pathology.

In the previous literature goblet cells have been identified by the presence of their secretory product using staining with PAS, the lectin HPA, or an antibody to MUC5AC.6 These techniques measure filled goblet cells. As goblet cells secrete their entire amount of secretory product, goblet cells that have secreted (empty cells) are not measured by these methods. In 1997 Krenzer and Freed found that cytokeratin 7 (CK7) stained the cell body of goblet cells.7 Thus it is now possible to identify the total goblet cell population, both total and filled, by

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Supported by Invest Northern Ireland (C.B.T.M., J.E.M.) and the Parkinson’s Disease Society (C.B.T.M., J.E.M.).

Submitted for publication December 8, 2010; revised March 17, 2011; accepted March 24, 2011.

Disclosure: J.E. Moore, None; G.T. Vasey, None; D.A. Dartt, None; V.E. McGilligan, None; S.D. Atkinson, None; C. Grills, None; P.J. Lamey, None; A. Leccisotti, None; D.G. Frazer, None; T.C.B. Moore, None

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using an antibody to CK7 along with the lectin HPA or an antibody to MUC5AC. Total goblet cells are CK7 positive, empty goblet cells are CK7 positive and HPA or MUC5AC negative, while filled goblet cells are CK7 positive and HPA or MUC5AC positive. A decrease in the total number of goblet cells indicates loss of cells. An increase in the total number of goblet cells would reflect goblet cell proliferation. A decrease in filled goblet cells would indicate secretion, whereas an increase in filled goblet cells would reflect inhibition of goblet cell secretion.

Reduced numbers of filled goblet cells, identified by PAS staining, has long been associated with dry eye syndrome and ocular surface inflammation. Gilbard et al.8 demonstrated that elevated tear film osmolarity is associated with decreased corneal glycogen, and others have reported a reduced number of filled conjunctival goblet cells.9 Other studies have further demonstrated the effect of hyperosmolarity on tear film instability in dry eye subjects,10 while other research has investigated the inflammatory cell signaling mechanisms involved in cultured corneal epithelial cells in response to hyperosmolar conditions.11

It is widely recognized that ocular surface inflammatory mediators can interact destructively contributing to or compounding damage in association with dry eye syndrome. Previous work12 demonstrated wide variation in results between commonly used clinical dry eye tests with little concordance found between tests in mild to moderate disease. While dry eye progression is known to be multifactorial, tear osmolarity has been demonstrated as the single best indicator of disease severity.13

This study seeks to assess both the effect of increased tear osmolarity alone (that is, tear osmolarity without evidence of other signs of ocular surface perturbation) as well as tear hyperosmolarity combined with composite increasing level of evidence of ocular surface perturbation on the goblet cell population.

METHODS

Study Participants
Research involving study participants adhered to the tenets of the Declaration of Helsinki. The research was approved by the Office of Research Ethics Committee in Northern Ireland (ORECNI). Study participants were recruited through poster advertisement in corneal clinics, dental clinics, and clinical referrals of patients attending corneal clinic presenting with signs and symptoms of ocular surface perturbation. The control grade consisted of normal volunteers, having no obvious signs or symptoms and no previous history of ocular surface abnormalities. Exclusion criteria included pregnancy and taking oral or topical antibiotics or prescribed eye medication. Patients were recruited on the basis of a positive result after McMonnies and short frequency questionnaires; however, a subsequent diagnosis was based on the finding of tear osmolarity > 308 mOsm/L. A further routine spectrum of tests was carried out on each patient (described subsequently). All patient results were subsequently analyzed for evidence of patterns of increasing composite levels of positive tests. Based on this analysis a pathologic classification was made from 0 to IV with normal represented by grade 0 and an abnormal ocular surface with increasing severity represented by grades I–IV.

Ocular Surface Assessments
Assessment of the tear film and ocular surface was performed in the following order: (1) biomicroscopic examination of the ocular surface, (2) lissamine green staining, (3) tear film break-up time (TFBUT) using fluorescein, (4) fluorescein staining, (5) Schirmer strip evaluation, and (6) conjunctival impression cytology. The sequence of testing performed constant for all subjects. One experienced optometrist performed all examinations and measurements throughout the study.

Classification of Ocular Surface into Grades 0–IV for Increasing Severity Based on Ocular Surface Pathology
Subjects were defined as having different grades of severity of ocular surface perturbation (grades 0–IV) based on a combination of tests, which took into consideration both the presence of hyperosmolarity and increasing levels of clinical evidence of ocular surface inflammation and other forms of recognized perturbations of the ocular surface. Grade 0 (normal) included all subjects with an osmolarity reading of < 308 mOsm/L, while hyperosmolarity was considered at > 308 mOsm/L14 and classified as grade I. Subsequent increasing levels of ocular surface perturbation, principally related to clinical evidence of ocular surface inflammation, combined with a hyperosmolarity of > 308 mOsm/L resulted in further grading. In summary, grade II included all subjects with hyperosmolarity > 308 mOsm/L plus conjunctival erythema ≥ 1, grade III included those with hyperosmolarity 308 mOsm/L plus conjunctival erythema ≥ 1 with lid inflammation ≥ 1, and grade IV included subjects with hyperosmolarity > 308 mOsm/L plus conjunctival erythema ≥ 1 with lid inflammation ≥ 1, meibomian gland disease ≥ 1, and conjunctival swelling ≥ 1.

Grade V (not present in this cohort) would have included subjects with hyperosmolarity > 308 mOsm/L plus conjunctival erythema ≥ 1 with lid inflammation ≥ 1 and meibomian gland disease ≥ 1 and conjunctival swelling ≥ 1 plus fluorescein staining of grades 3 and above and lissamine green staining of grades 3.

Meibomian Gland and Ocular Surface Grading
A biomicroscope examination of the meibomian glands, lids, conjunctiva, and tear film was performed at a slit lamp to grade the presence/ severity of meibomian gland disease (MGD) and assess signs of ocular surface abnormality and inflammation. The grading scale was categorized according to that previously described.15 For the purpose of statistical analysis, MGD grades 3 or above, fluorescein staining of grades 3 and above, and lissamine green staining of grades 3 are indicative of severe composite clinical grade V.16

Measurement of Tear Osmolarity
Osmolarity was measured using a laboratory-on-a-chip to simultaneously collect and analyze the electrical impedance of a 50 nanoliter tear sample from the inferior lateral meniscus (TearLab Osmolarity System; TearLab Corporation, San Diego, CA). The highest level of either eye was taken to represent the true value.

A brief description of the additional tests carried out to characterize the ocular surface for this study is outlined below.

Dry Eye Symptoms
All participants completed a McMonnies dry eye questionnaire17 consisting of 14 questions and a possible score of 0–45. Any score of ≥ 14 has previously been recommended as indicative of a dry eye diagnosis.18–20 The questionnaire included questions about age, dry eye treatments, environmental factors, and medication usage and assessed the presence of six dry eye symptoms (soreness, dryness, grittiness, itching, stinging, and burning). A second, short frequency questionnaire (SFQ) was also completed by all participants consisting of three questions: two assessing symptoms (dry and irritated) and one with a yes or no for previous dry eye diagnosis.

Schirmer Test
Tear volume was assessed (Schirmer Tear Test Strips; Haag-Streit, UK Ltd, Harlow, UK). Topical anesthesia was instilled to the eyes, and the conjunctival fornix was dried with a cotton tip applicator. After a 2-minute waiting period the strip was placed into the lower conjunctival sac of both eyes, in the temporal region, for 5 minutes, and the
TABLE 1. Cytological Grading of Conjunctiva Using PAS Stained Impression Cytology Samples

<table>
<thead>
<tr>
<th>Grade</th>
<th>Four High-Power Fields of View</th>
<th>Stratified Squamous Cell Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;30 goblet cells</td>
<td>Small round epithelium with nucleocytoplasmic ratio of 1:2</td>
</tr>
<tr>
<td>2</td>
<td>15–30 goblet cells</td>
<td>Larger polygonal epithelial cells and nucleocytoplasmic ratio of 1:3</td>
</tr>
<tr>
<td>3</td>
<td>5–15 goblet cells</td>
<td>Decreased nucleocytoplasmic ratio</td>
</tr>
<tr>
<td>4</td>
<td>&lt;5 goblet cells</td>
<td>Large epithelial cells with pyknotic nuclei visible</td>
</tr>
</tbody>
</table>

TABLE 2. Grading of Total Goblet Cell Number Using CK7 Staining

<table>
<thead>
<tr>
<th>Grade</th>
<th>Goblet Cell No. per HPF (×4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;30</td>
</tr>
<tr>
<td>2</td>
<td>15-30</td>
</tr>
<tr>
<td>3</td>
<td>5-15</td>
</tr>
<tr>
<td>4</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

Keynes, UK) at 400× magnification. Negative control reactions consisted of substituting PBS for the primary antibody.

Total goblet cell number was assessed by calculating the number of CK7+ goblet cells in 1 HPF (40×) as per Table 2. To equilibrate and use the same numbers grading as used for PAS staining, all CK7+ numbers were multiplied times 4.

Statistical Analysis

Patients were recruited on the basis of a positive result after taking McMonnies and short frequency questionnaires. Subsequent data collected was filtered; samples with missing variables were removed from the study for the purpose of comparison and completeness. Goblet cell number and function were ascertained by assessing the presence or absence of PAS, CK7, and HPA in cells on impression cytology samples taken from the ocular surface of all subjects. Grading from 1 to 4 was defined whereby increasing grade reflected lower numbers of goblet cells or increasing number of empty goblet cells. We calculated the descriptive statistics for our variables and visually represented the relationships of interest in bar charts or scatter plots including lines of best fit to show trends in the data. We used Pearson’s correlation coefficient to calculate the strength of relationships between variables. Data assessed for evidence of correlation included tear osmolarity and PAS, CK7, and HPA grading. For the composite clinical pathologic grade we used osmolarity scoring along with the addition of increasing severity indicators (conforming with one DEWS purported mechanism: inflammation) as the group numbers increased (grading system 0–IV).

In addition, when osmolarity was compared to the various grades we also used the Kruskal Wallis test, which allowed for the comparison between ordinal and continuous data. Moses’ test of extreme reactions was used to calculate if the range of results was different for goblet cell numbers and if the ratio between filled and empty goblet cells was different for high-grade samples compared with lower-grade samples.

RESULTS

Initial assessment for correlation between tear osmolarity and goblet cell number and function demonstrated no significant correlation nor threshold effect for these singular parameters.

Regression analysis for tear osmolarity with total goblet cell number (CK7+) returned a $R^2$ value of 0.009; with filled goblet cell number (CK7+ + HPA+) returned a $R^2$ value of 0.016; and with empty goblet cell number (CK7+ + HPA-) returned a $R^2$ value of 0.009. Thus, the lack of an $R$ value close to zero would suggest no significant correlation.

Helix Pomatia Agglutinin Lectin (HPA) and Cytokeratin 7 (CK) Immunofluorescence Microscopy Analysis of Impression Cytology Samples

Impression cytology samples fixed with ethanol were examined for the presence of CK7 to indicate the cell bodies of goblet cells independent of secretory product and HPA to indicate secretory product within goblet cells. Briefly, the slides were washed in phosphate-buffered saline (PBS) and blocked in 1% BSA and 0.2% Triton-X in PBS for 30 minutes at room temperature. Slides were then incubated with HPA or PAS directly conjugated with Alexa Fluor 488 (1:100; Invitrogen, Paisley, UK) and CK7 (1:5; MP Biomedicals, Livingston, Scotland) in PBS for 1 hour at room temperature. Samples were then incubated with 1:50 dilution of a TRITC conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour. Finally, slides were washed in PBS before being mounted between two coverslips in DAPI mounting media (Santa Cruz Biotechnology). All samples were visualized and counted using an SP5 confocal/multiphoton microscope (Leica, Milton Keynes, UK) at 400× magnification. Negative control reactions consisted of substituting PBS for the primary antibody.
to 1 indicated that no evidence of correlation was found between tear osmolarity and goblet cell number and function as measured by any of the parameters above in this cohort.

Using a Pearson correlation coefficient no statistical evidence of threshold effect was found between tear osmolarity and either total, empty, or full goblet cell number. More than 40 different thresholds were systematically assessed from >275 mOsmol/L to >360 mOsmol/L; no statistical significance was reached when a statistical significance was set at \( P \leq 0.05 \).

**Relationship between Tear Osmolarity Level and Composite Clinical Dry Eye Grading (Grades 0–IV) Ascertained Using Ocular Surface Pathology**

All subjects were graded into grades 0–IV to reflect the increasing levels of ocular surface perturbation combined with the hyperosmolarity reading of \( \geq 308 \) mOsm/L as the definition of grade 0 was an osmolarity \( < 308 \) mOsm/L and as grades I–IV were \( \geq 308 \) mOsm/L; grade 0 was not formally assessed for statistical significance. The mean osmolarity value for each grade (I–IV) was compared, and no statistically significant increase in the mean grade osmolarity was noted between any of the lower composite pathologic grades of I, II, or III (Fig. 1). The mean osmolarity per group for grades 0–III was 297.9, 323.3, 319.2, and 323.8 mOsm/L, respectively. In contrast, grade IV subjects, overall as a group, showed a statistically significant increase in mean tear osmolarity with a value of 344 mOsm/L compared with all other groups \( (P = 0.000) \); in both correlation tests and the Kruskal-Wallis test. Increased tear osmolarity was associated with degree of severity for only the more severe group of subjects (grade IV), which in this study had the equivalent of moderate dry eye.

The average TFBUT and Schirmer’s for each pathologic grouping varied as shown in Table 3. There was a significant reduction in TFBUT for group IV compared with group 0, but no other clear pattern was evident for the other groupings for either TFBUT or Schirmer’s test. Thus, these parameters were not considered in the pathologic grading of severity. Additionally no linear correlation was found between TFBUT and goblet cell numbers, nor was there any evidence of threshold effect between TFBUT and goblet cell numbers or functions (empty or full goblet cell numbers/ratio).

**Relationship between composite clinical pathologic grading (Grades 0–IV) and Total Goblet Cell Number as Measured by CK7**

Total goblet cell number was defined as number of CK7-positive goblet cells per unit area and included both filled and unfilled goblet cells. Goblet cell density did not change with increasing severity of disease from grades 0 to III (Fig. 2). Goblet cell density decreased significantly at the most severe clinical pathologic grade (grade IV). Using Moses’ test this decrease was significant with \( P = 0.000 \). A decrease in the total number of goblet cells was associated only with the most severe grade, which in this study was equivalent to moderate dry eye.

**Relationship between Goblet Cell Population Ascertained through HPA and CK7 Levels and Composite Clinical Grading (Grades 0–IV)**

The number of filled and empty goblet cells, as well as the ratio of filled:empty goblet cells, was assessed by HPA and CK7 staining. The number of filled goblet cells was indicated by cells positive for both HPA and CK7. The number of empty goblet cells was indicated by cells, which were HPA negative but CK7 positive. The number of total goblet cells was significantly lower in grade IV compared with all other grades (Fig. 3A). There was also a trend, which did not, however, reach statistical significance, of reducing number of filled goblet cells compared to unfilled from grades 0–III. This was demonstrated as a gradual decrease in ratio of filled:empty goblet cells with increasing grade from grade 0 to III (Fig. 3B). In contrast, at grade IV there was a significant increase in the ratio of filled:empty goblet cells compared with grades 0–III. The change in ratio was found to be significant with a Kruskal-Wallis significance level of \( P = 0.011 \), and Moses’ test returned a highly significant \( P \) of 0.000. Note this decrease in the number of total goblet cells

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933460/)  
**FIGURE 1.** Relationship between tear osmolarity level and composite clinical pathologic grading (grades 0–IV) ascertained using ocular surface pathology. Tear osmolarity for all subjects in each grade was expressed as mean \( \pm \) SEM and plotted versus the dry eye grade. The number of subjects was 45 in grade 0, 28 in grade I, 14 in grade II, 6 in grade III, and 6 in grade IV (total 99). With grade IV there was a statistically significant increase in the mean osmolarity value compared with that of all the lower grades (\( \geq 344 \) mOsm/L, \( P = 0.000 \); in both correlation tests and the Kruskal-Wallis test.

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933460/)  
**FIGURE 2.** Relationship between composite clinical pathologic grading (grades 0–IV) and total goblet cell number as measured by CK7. Total goblet cell number was defined as number of CK7-positive goblet cells per unit area and included both filled and unfilled goblet cells. Goblet cell density did not change with increasing severity of disease from grades 0 to III (Fig. 2). Goblet cell density decreased significantly at the most severe clinical pathologic grade (grade IV). Using Moses’ test this decrease was significant with \( P = 0.000 \). A decrease in the total number of goblet cells was associated only with the most severe grade, which in this study was equivalent to moderate dry eye.

<table>
<thead>
<tr>
<th>Group</th>
<th>TFBUT (seconds)</th>
<th>Schirmer’s Test (mm Wetting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.873</td>
<td>9.292</td>
</tr>
<tr>
<td>I</td>
<td>7.479</td>
<td>12.061</td>
</tr>
<tr>
<td>II</td>
<td>5.925</td>
<td>7.071</td>
</tr>
<tr>
<td>III</td>
<td>6.74</td>
<td>10.8</td>
</tr>
<tr>
<td>IV</td>
<td>3.867</td>
<td>10.167</td>
</tr>
</tbody>
</table>

**TABLE 3.** Average TFBUT and Schirmer’s Stratified by Clinical Pathological Grade

![Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933460/](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933460/)
and increase in the ratio of filled to empty goblet cells was associated with only the most severe grade, which in this study was equivalent to moderate dry eye. It must be recognized that the numbers in this group are relatively small (n / H_11005 6).

**Correlation Analysis of Tear Osmolarity and Total Goblet Cell Number as Indicated by CK 7 Positivity**

CK 7 staining was used to delineate total goblet cell numbers for the subjects recruited to this study. A regression line was plotted for tear osmolarity values versus the total number of goblet cells (Fig. 4). Figure 4 demonstrates the negative trend found between increasing tear osmolarity and increasing number of total goblet cells did not reach statistical significance at the preset significance level of P = 0.05. Correlation analysis produced a negative coefficient of -0.106.

**Relationship between Tear Osmolarity Level and Histopathological Grading Using Total Goblet Cell Number**

CK 7 data, which indicate total number of goblet cells (goblet cell density), were used to categorize subjects into grades of increasing levels of severity. Briefly, subjects were assigned to grades 1 to 4 dependent on the number of cells positive for CK 7, i.e., goblet cell number per four high-power fields: grade 1, n = >30; grade 2, n = 15–30; grade 3, n = 5–15; and grade 4, n = <5 goblet cells. This scoring system indicates increasing severity of goblet cell loss. As CK7 grading increased in severity from grade 1 to 3, the tear osmolarity did not change remaining at an average of 310 mOsm/L for grades 1, 2, and 3 respectively (Fig. 5; see also Table 2). The highest grade of severity, grade 4, demonstrated a statistically significant increase in tear osmolarity, which was 330 mOsm/L. This osmolarity value was significant at P = 0.024, derived from a positive correlation coefficient of 0.227, compared with all other grades 0 to 3 (Fig. 5). Increased tear osmolarity was associated with decreased number of total goblet cells only for the most severe level of goblet cell loss, which in this study was equivalent to moderate dry eye.

**Figure 3.** Relationship between goblet cell function ascertained through number of HPA and CK7 labeled cells and composite clinical pathologic grading (grades 0–IV). The number of filled (HPA +, CK7+) and empty (HPA−, CK7+) was expressed as mean ± SEM for each grade of composite clinical dry eye (A). The ratio of filled: empty goblet cells was determined for each grade of composite clinical grading (B). The number of subjects was 45 in grade 0, 28 in grade I, 14 in grade II, 6 in grade III, and 6 in grade IV (total 99). The ratio of filled to empty goblet cells for grade IV was found to be significant with a Kruskal-Wallis significance level of P = 0.011, and Moses’ test returned a highly significant P of 0.000.

**Figure 4.** Correlation analysis of tear osmolarity and total goblet cell number as indicated by number of CK 7+ cells. CK 7 staining was used to delineate total goblet cell numbers for the 99 subjects. A regression line was plotted for tear osmolarity values versus the total number of goblet cells. The negative trend found between increasing tear osmolarity and increasing number of total goblet cells did not reach statistical significance at the preset significance level of P = 0.05. Correlation analysis produced a negative coefficient of -0.106.

**Figure 5.** Relationship between tear osmolarity level and composite histopathological grading ascertained using total goblet cell number (grades 1–4) determined by number of CK 7+ cells. Tear osmolarity was expressed as mean ± SE and plotted versus a goblet cell grading system using number of CK 7+ positive cells (total number of goblet cells) described in Table 2. A statistically significant increase in osmolarity within the highest grade 4 at 330 mOsm/L significant at P = 0.024, derived from a positive correlation coefficient of 0.227, compared to all other grades 1–3.
Effect of Tear Osmolarity on Goblet Cell Profile

Figure 6. Relationship between tear osmolarity level and composite histopathological grading determined from number of PAS-stained goblet cells and stratified squamous cells metaplasia (grades 1–4). Tear osmolarity was expressed as mean ± SEM and plotted versus a goblet cell grading system using number of PAS positive goblet cells and degree of squamous metaplasia shown in Table 1. The higher the PAS grade the lower the number of filled goblet cells. Statistically significant increase in mean tear osmolarity for the higher grades of goblet cell loss, grades 3 and 4, 321 and 320 mOsm/L respectively, was significant at P = 0.023.

Relationship between Tear Osmolarity and Histopathological Grading Based on PAS Staining of Conjunctival Epithelial Cells

Grading using PAS staining was used to assess changes in the number of filled goblet cells (PAS+) and degree of squamous metaplasia of the stratified squamous cells. The grading system is shown in Table 3. As demonstrated in Figure 6, PAS grades 1 and 2 both had similar tear osmolarity values of 309 and 311 mOsm/L, respectively. For the more severe grades, 3 and 4, there was a statistically significant increase in tear osmolarity. Tear osmolarity for grades 3 and 4 increased to 321 and 320 mOsm/L, respectively, and was significant at P = 0.023. Increased tear osmolarity was associated with decreased number of filled goblet cells and increased degree of squamous metaplasia for the two most severe levels of PAS grade. PAS grade was the only variable for which two grades of severity rather than only the most severe grade alone was associated with an increase in tear osmolarity.

Discussion

Goblet cells perform an important function at the ocular surface by producing and secreting soluble mucin into the tear film. It has long been recognized that filled goblet cells are lost early in dry eye disease. Grading using PAS staining was used to assess changes in the number of filled goblet cells (PAS+) and degree of squamous metaplasia of the stratified squamous cells. The grading system is shown in Table 3. As demonstrated in Figure 6, PAS grades 1 and 2 both had similar tear osmolarity values of 309 and 311 mOsm/L, respectively. For the more severe grades, 3 and 4, there was a statistically significant increase in tear osmolarity. Tear osmolarity for grades 3 and 4 increased to 321 and 320 mOsm/L, respectively, and was significant at P = 0.023. Increased tear osmolarity was associated with decreased number of filled goblet cells and increased degree of squamous metaplasia for the two most severe levels of PAS grade. PAS grade was the only variable for which two grades of severity rather than only the most severe grade alone was associated with an increase in tear osmolarity.

Lectin HPA has been shown to be an indicator of secreted protein including the mucin MUC5AC within ocular surface goblet cells. In this particular study the presence or absence of HPA within goblet cells colabeled with CK7 was used as an indicator of filled or empty goblet cell status. This study showed that irrespective of whether staining with CK7 (Figs. 2 and 3) or with PAS (Fig. 6) that the overall number of goblet cells was reduced significantly with the higher clinical pathologic grades. The data enabled us to create a simple composite grading system for each parameter measured, focusing on clinical markers of inflammatory pathology to enable us to assess the increasing stepwise effect of adding pathologic inflammatory clinical signs resulting in increasing grades of severity. We then assessed the association between these increased composite pathologic grades and mean tear osmolarity with goblet cell number and function.

Ocular surface inflammation can be present secondary to dry eye pathology or may be present independent of or in conjunction with dry eye syndrome. The goblet cell population on the ocular surface appears to have the ability to tolerate isolated tear hyperosmolarity, as evidenced by our findings from Figure 4 and our assessment of threshold levels of tear osmolarity at least for the range of tear osmolarity within this cohort (275–375 mOsmol/L) irrespective of threshold. The findings from this study would suggest that when tear hyperosmolarity is present (i.e., >308 mOsmol/L) the goblet cell population may remain capable of tolerating clinical evidence of increasing ocular surface inflammation and any associated pathology only until a certain threshold as defined in this particular study by the composite pathologic grade IV (Figs. 2, 3, and 4). This combination of composite clinical evidence of more widespread ocular surface inflammation that may be deleteriously affecting the goblet cell population (Figs. 2, 3, and 4) is not unexpectedly also associated with a raised tear osmolarity level (Fig. 1).

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The relationship between these factors is not a simple linear continuous relationship. Similarly no specific single threshold effect was found between tear osmolarity and goblet cell pathology, despite systematically assessing more than 40 consecutive levels of tear osmolarity between 275 and 375 mOsmol/L. A similar lack of correlation was found between FTBUT and goblet cell number or function and similarly no distinct threshold effect was found. Statistically significant correlation between osmolarity changes and histopathological evidence of reduced goblet cell numbers as seen through PAS or CK7 staining in this study therefore did not occur in isolation rather only occurred in conjunction with other evidence of ocular surface inflammation or perturbation as indicated by the higher clinical pathologic grade IV. These findings would therefore appear to indicate that in mild to moderate dry eye disease (as defined by DEWS1) hyperosmolarity singularly does not correlate well with pathologic changes within the ocular surface goblet cell population. Its association with decreased number of goblet cells in this study was shown only to occur, as part of a greater multifactorial disease process. Further investigation is required to elucidate key molecular biological factors involved in tipping the balance of the ocular surface from healthy homeostasis into pathologic change and detrimental effects on the goblet cell population.

References