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Development of a new grading scale for tear ferning

Ali M. Masmali[a,d,*], Paul J. Murphy[b], Christine Purslow[c,d]

[a] Cornea Research Chair (CRC), Optometry Department, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia
[b] School of Optometry and Vision Science, University of Waterloo, Waterloo, Canada
[c] School of Health Professions, Plymouth University, Plymouth, UK
[d] Contact Lens and Anterior Eye Research (CLAER) Unit, School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK

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ABSTRACT

Purpose: This paper reports on the development of a new tear ferning (TF) subjective grading scale, and compares it with the Rolando scale.
Method: TF patterns obtained from tear film samples collected from normal and dry eye subjects in previous studies were collated into a large image library. From this library, 60 images were selected to represent the full range of possible TF patterns, and a further sub-set of 15 images was chosen for analysis. Twenty-five optometrists were asked to rank the images in increasing order between extreme anchors on a scale of TF patterns. Interim statistical analysis of this ranking found 7 homogeneous sub-sets, where the image rankings overlapped for a group of images. A representative image (typically the mean) from each group was then adopted as the grade standard. Using this new 7-point grading scale, 25 optometrists were asked to grade the entire 60 image library at two sessions: once using the 4-point Rolando scale and once using the new 7-point scale, applying 0.25 grade unit interpolation.
Results: Statistical analysis found that for the larger image set, the Rolando scale produced 3 homogeneous sub-sets, and the 7-point scale produced 5 homogeneous sub-sets. With this refinement, a new 5-point TF scale (Grades 0–4) was obtained.
Conclusions: The Rolando grading scale lacks discrimination between its Type I and II grades, reducing its reliability. The new 5-point grading scale is able to differentiate between TF patterns, and may provide additional support for the use of TF for both researcher and clinician.
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1. Introduction

The chemical analysis of tear film composition is difficult due to the small volumes available, and to the transparent and dynamic nature of tears [1]. Clinicians and scientists recognise that biochemical analysis of osmolarity and other key components in a tear sample is the way forward, but the small volumes involved make biochemical analysis particularly challenging [2,3]. Techniques available are limited by the need for expensive equipment that is difficult to use under normal clinical conditions [4]. A simple, clinical tear film test, that is quick and inexpensive to perform, and can indicate the biochemical properties of the tear film, would be very useful.

One potential and clinically suitable test involves drying a tear sample on a glass microscope slide to produce a crystallisation pattern in the form of a fern [5–7]. This phenomenon occurs with many body fluids and follows a characteristic formation process. The first discovery of tear crystallisation was reported by Fourcroy and Vauquelin in 1791 [8], but remained unnoted until 1946, when observed by Papanicolaou while studying cervical mucus [9]. Ferning patterns have been used to test different body fluids, such as vaginal and cervical mucus as an indicator of the menstrual cycle [10], oestrogen activity and ovulation [11–14] and early pregnancy [13,15]. Ferning has also been used to test saliva [16], to consider the observation of salivary ferning as a new technique for determining the fertile period [17], and to correlate salivary ferning and the fertile period [18], and using of salivary ferning in ovulation detection in family planning [19].

Crystallisation begins with the formation of a nucleus, consisting of a regularly arranged number of ions. The nucleus is formed by aggregation when the solute evaporates and dissolved ions are concentrated until super-saturation of the tear film is reached [7]. The nucleation process begins at the peripheral edges of the drop, where the solution is thinnest and super-saturation is reached rapidly [7]. Each nucleus has the ability to grow into a large crystal unit with the addition of more ions, and, so long as the sample solute is able to diffuse into areas with a lower solute concentration area, normal crystals can form. This requires a slow growth rate, low solution viscosity and low impurity levels to permit free solute diffusion.

* Corresponding author at: Cornea Research Chair (CRC), Optometry Department, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia. Tel.: +966 11 4693547; fax: +966 11 4693536.
E-mail address: amasmali@ksu.edu.sa (A.M. Masmali).

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The absence of these conditions can lead to dendritic crystal growth [20]. In this situation the stems grow longer and branch at regular intervals along the main stem. The reason for this regularity is not understood [7], but it is known that fern-like dendritic growth can be promoted by increasing the evaporation rate of the drop, by reducing atmospheric humidity, by increasing the drying temperature, or when impurities are present in low concentration, which acts as additional nuclei for crystal deposition [7].

Since tears are a complex solution, with many organic and non-organic components, the tear fern pattern produced by drying a sample depends on the composition of the tear sample [4,7]. This variation in pattern has been suggested as a simple test for tear film quality at a gross biochemical level. This phenomenon gives tear ferning the potential, and the features, to be used as a diagnostic test in the clinic [5,21]. Previous studies have demonstrated it to show good repeatability [22], sensitivity and specificity [21,23,24].

Different scales for grading tear ferning patterns have been proposed [6,21,25] with the Rolando scale being adopted as the main method used in previous published work in this area. However, the Rolando scale was not originally developed to produce a repeatable, standardised grading instrument, rather it arose from Rolando's observation that the Type I and II patterns were found in the majority of normal eyes, while Types III and IV were found in the majority of keratoconjuctivitis sicca (KCS) eyes [6].

The main difficulty with using the Rolando scale lies with this gross categorisation of ferning patterns, restricting sensitivity – the variance around Types I and II is particularly large – and not all types of tear ferning patterns are represented by the scale [22]. If the tear ferning test is to become part of routine clinical examination of the tear film, it is important to have a grading scale that has been developed to meet the needs of the clinician, and to address the four fundamental design requirements of a grading scale [26].

The aim of this paper is to report on the development of an improved subjective grading scale for clinicians, and the comparison of the new subjective scale with the Rolando scale.

2. Methods

A digital image library was compiled from tear ferning patterns produced using a standardised protocol, all images were observed under digital microscope (Leica DMRA2) with 10× magnification, and all images were saved in JPEG file format [22]. In total, 560 images of tear ferning patterns were produced from tear samples collected from 157 subjects, and all images were graded to 0.25 increments of the Rolando scale, for increased sensitivity [26]. Sixty images were selected by the authors, according to Rolando's grading scale, to be representative of the full range of possible tear ferning patterns.

From the 60 image library, 15 images were further selected to represent the range of tear ferning patterns. Fifteen was judged to be a workable number for clinicians to rank at a single session in an experimental setting. Although the Rolando scale was used to assist in selecting an equal number of images across the range, this was a notional attribute used only to help in image selection.

Twenty-five experienced optometrists working in the School of Optometry and Vision Sciences at Cardiff University were presented with hard copies of the fifteen images and asked to rank the fifteen images in ascending order between two 'anchors' – Reference 1 (a densely branched Rolando Type I) to Reference 2 (a sparse Rolando Type IV). Each image had the same magnification (10×) and was printed to the same size (12 cm × 10 cm), then labelled with two random capital letters and laminated. Each volunteer was given a record sheet, with a numeric table from 1 to 15, on which they recorded the alpha-code of each image in the rank order they felt best matched the pattern progression between the two references images. There was no time limit given and each volunteer was reminded that there was no right or wrong ranking, only his or her opinion. A value (weighting) was assigned to each position in the ranking (i.e. position 1 was worth 1 point, position 2 worth 2 points, position 7 worth 7 points). This produced 25 weighted rankings for each image, and the average (and variance) weighting for each image was calculated (Table 1). The data was normally distributed (Kolmogorov–Smirnov; $p > 0.05$). A one-way ANOVA was used to compare the score weightings attributed to each image, and a statistically significant difference ($p < 0.0005$) was observed. Post hoc Tukey HSD testing revealed seven homogeneous sub-sets, within which no statistically significant differences were found (Table 2).

Table 1
The average position score for each image.

Image	Sum of score	Mean score	SD
1	107	4.28	2.98
2	101	4.04	2.94
3	97	3.88	1.96
4	86	3.44	2.22
5	140	5.6	2.10
6	117	4.68	1.70
7	124	4.96	2.17
8	159	6.36	1.89
9	221	8.84	1.25
10	247	9.88	1.72
11	263	10.52	1.50
12	287	11.48	2.20
13	329	13.16	0.37
14	349	13.96	0.54
15	372	14.88	0.33

The seven groups, representing the homogeneity amongst the 15 images, supported the strategy to use a single image from each group to represent the library: a new 7-item scale. The mean score of the images in each sub-set was used to select a representative image (Table 3), and the image score closest to the mean was chosen to be representative of the sub-set (Table 4). This produced seven images, selected to represent a new 7-point tear ferning grading scale (Fig. 1).

This new scale was then validated against the larger sample of sixty images. Twenty-five optometrists experienced in clinical grading attended the laboratory for two sessions. Each observer was asked to grade all sixty library images displayed via a random slide-show presentation (Microsoft PowerPoint). The images were displayed on the screen under identical luminance and resolution (screen size 13.3 in., and resolution of 1280 × 800

Table 2
Seven homogeneous sub-sets were found using post hoc Tukey HSD test; the table shows the mean weighting for the homogeneous sub-sets.

Image	*N*	Sub-set for alpha = 0.05						
		1	2	3	4	5	6	7
4	25	3.44						
3	25	3.88	3.88					
2	25	4.04	4.04					
1	25	4.28	4.28					
6	25	4.68	4.68	4.68				
7	25	4.96	4.96	4.96				
5	25		5.60	5.60				
8	25			6.36				
9	25				8.84			
10	25				9.52			
11	25				10.52	10.52		
12	25					11.48	11.48	
13	25						13.16	13.16
14	25							13.96
15	25							14.88
Sig.		0.278	0.118	0.143	0.143	0.920	0.143	0.118

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