



Application of a new grading scale for tear ferning in non-dry eye and dry eye subjects



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ABSTRACT

Purpose: To apply the Masmali tear ferning (TF) grading scale on non-dry eye (NDE) and dry eye (DE) subjects to test the validity of the grading scale in practice, and to describe the grading scale range for NDE and DE.

Method: Forty NDE subjects (20 males, 20 females) and 40 DE subjects (23 males, 17 females) ranging in age from 19 to 53 years (mean \pm SD: 25.3 ± 5.5) with no other ocular disease, no contact lens wear, and not pregnant or breastfeeding were recruited. McMonnies scores were used for subject grouping. Phenol red thread (PRT) and slit-lamp test were used. A tear sample was collected from right eye, which was then dried to produce a ferning pattern, that was observed using a digital microscope, and graded.

Results: Mean McMonnies, PRT and TF grade in NDE subjects were 7.1 ± 3.8 , 27.4 ± 4.3 mm and 0.78 ± 0.40 , respectively. Median McMonnies, PRT and TF grade in DE subjects were 16.5 ± 3.0 , 9.0 ± 2.0 mm and 2.3 ± 1.48 , respectively. In NDE subjects, grades 0.0–1.8 were observed (82.5% Grade 0.0–1.00). Grades 2.0–4.0 were observed in DE subjects (72.5% Grades 2.0–3.0). For all subjects, there were large correlations between TF grade and PRT ($r = -0.79$), PRT and McMonnies ($r = -0.60$), and TF and McMonnies ($r = 0.73$).

Conclusions: The Masmali TF grading scale showed good validity in describing the TF patterns. Grades ≥ 2 can be classified as abnormal patterns. The TF test has the potential to be used in the clinic.

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1. Introduction

The tear film has three main functions: maintenance of ocular surface health, creation of a smooth optical surface for clear vision, and protection of the eye from foreign bodies and infection. It consists of three main layers [1–4]. The outermost layer of the tear film is the lipid layer. This layer acts to limit evaporation of the aqueous component, as well as providing lubrication for eyelid movement and a smooth optical surface [5]. The middle layer consists principally of water, which contains ions and nutrients to supply the corneal and conjunctival epithelia, as well as immunological proteins for ocular defence against infections [6]. The innermost layer consists of mucous secreted from conjunctival goblet cells and from the corneal epithelia, which provides a

foundational glycocalyx over the ocular surface to which the tear film can adhere [2].

Dry eye (DE) is a multifactorial disease of the ocular surface and the tears that interferes with the normal production and function of the tear film, and which leads to decreases in tear stability and tear volume, and increases in evaporation and tear osmolarity [7,8]. It can also lead to damage to the ocular surface, visual disturbance, and discomfort [8,9]. It has been reported that DE can affect quality of life in different ways. Symptoms of irritation, effects on visual function or performance are all signs and symptoms that can affect quality of life of DE patients [8]. Blurry vision, hyperaemia, mucoid discharge, ocular irritation and dryness are the most common complaints associated with DE [8]. The diagnosis of DE is complicated due to the multifactorial etiology. In particular, the small tear film volume limits potential analysis of the tear film composition, which may contain immunological markers for DE [8]. Tear film composition analysis is also very challenging for clinicians and researchers because of the dynamic and transparent nature of tears [10–12]. Various methods have been developed to test some aspect of the

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tear film, such as Schirmer's test [13–15], phenol red thread (PRT) test [16–19], tear osmolarity [20–23], tear meniscus height [24,25] and tear break-up time (TBUT) [26]. Each test assesses some aspect of the tear film, but no single test is able to definitively diagnose dry eye. Instead a combination of tests is employed to provide a final diagnosis [8].

In defining dry eye, the Dry Eye Workshop reports (2007) [8], emphasised the role of tear osmolarity within a model of the disease process cycle. Due to either an under-production of tears or to increased tear evaporation, the salt concentration of the tear film increases, creating an osmotic stress for the ocular surface epithelia. This leads to an inflammatory response for the ocular surface, which further alters the tear film, leading to a reinforcing cycle of increasing osmolarity and inflammation [8]. Analysis of the tear osmolarity is therefore a priority for dry eye disease management, and the TearLab Osmolarity System (TearLab Corporation, San Diego, USA) uses 'lab on a chip' technology to provide an in-clinic assessment [27].

A potential alternative to instrument-based osmolarity testing is to use the phenomenon of tear ferning. Tear ferning involves the drying of a tear sample on a glass slide at normal room temperature and humidity conditions to produce a fern, a crystallization pattern that can be inspected by the use of a light microscope [28–30]. The test can be performed within the clinic quickly and cheaply with the advantage that the tear film chemical properties, especially electrolytes and large molecules, like proteins, can be so investigated indirectly [31]. Since the tear film is a complex solution and has different organic and inorganic components, variation in its composition or concentration will produce changes to the tear fern pattern [30,32].

The pattern variation has been suggested as a simple test for the quality of the tear film, with the potential of being used in the optometrist's clinic [28,33] and has shown good sensitivity and specificity [33–35], and repeatability [31]. To assist in assessing the ferning patterns, Rolando developed a tear ferning pattern grading scale [29]. The Rolando scale categorises the observed tear ferning pattern into four grades, known as Type I, II, III and IV [29]. It was found that Types I and II were common within healthy eyes and Types III and IV were common within kerato-conjunctivitis sicca eyes [29]. Very recently, a new grading scale for the tear ferning pattern has been developed to overcome some of the limitations associated with Rolando scale, such as the overlap between grades [31,36]. The new five-point Masmali grading scale can be used by both researcher and clinician when using the tear ferning test to investigate dryness of the eye.

Grade 0 has the full phenomenon of ferning pattern, with no spaces or gaps between the ferns and branches; the density of ferns and branches is decreased in Grade 1 with the appearance of small spaces and gaps between the ferns and branches. These ferns and branches are decreased to become thick and large with the presence of clear spaces and gaps in Grade 2. The spaces and gaps are increased in Grade 3 with no ferns, but with the presence of large crystals. The phenomenon of ferning pattern is totally absent in Grade 4. A visual presentation of the five grades has been published in Masmali et al. [31].

The aim of this research was to apply the new Masmali TF grading scale on tear samples from subjects with NDE and DE to test the validity of the grading scale in practice, and to describe the grading scale range for NDE and DE for the first time using this scale.

2. Methods

Eighty subjects (40 non-dry eyes (NDE): 20 males and 20 females; 40 dry eyes (DE): 23 males and 17 females), who ranged

in age from 19 to 53 years (mean \pm SD: 25.3 ± 5.5 years) with no symptoms of any other ocular disease, who did not wear contact lenses, and were not pregnant or breastfeeding, were enrolled in the study. All subjects completed a McMonnies dry eye symptoms questionnaire and were grouped into a healthy or dry eye group according to their response to the McMonnies questionnaire. Dry eye was diagnosed for a score >14.5 [37,38].

Slit lamp assessment of the ocular surface and adnexa was performed first, followed by measurement of tear volume with the phenol red thread (PRT) test were further used to describe the two subject groups. The slit-lamp examination was performed to check the external and anterior part of the eye for the absence of any ocular disease.

PRT strips were purchased from ZONE-QUICK (Showa Yakuhin Kako Co., Ltd). A 3-mm length of the thread was folded and inserted 1/3 of the distance from the temporal canthus of the lower eyelid, with the eye in the primary position. The thread was gently removed after 15 s and the length of the discoloured portion was determined (mm). The test was applied to the both eyes, but the reading for the right eye only was used in analysis.

Tear samples (1 μ l) were collected from the lower meniscus of the right eye using glass capillary tubes (10 μ l, Drummond Scientific Company, USA) and allowed to dry on a clean, unused glass slide for 10 min under normal room temperature (23 °C) and humidity (40%). Samples were immediately observed under digital microscope (Olympus DP72) with 10 \times magnification. Each ferning pattern observed was graded using the five-point grading scale [31] in 0.1 increments to improve grade refinement [39].

The study design was masked to avoid any bias. The McMonnies questionnaire, slit-lamp examinations, PRT and tear collections were completed by one investigator, and the imaging of the tear ferning patterns slides and the grading of the ferning patterns was completed by another investigator who was blind to the subject dry eye classification and other test results. All subjects were examined at a morning visit between 8:30 and 11:30 am.

Ethical approval was obtained from the College of Applied Medical Science Research Centre, King Saud University. The research followed the tenets of the Declaration of Helsinki, in which informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study.

2.1. Statistical analyses

Data were collated using Excel (Microsoft Office 2010) and analysed using the SPSS software (IBM Software, version 20). Data of tear ferning, PRT and McMonnies were examined for normality using Kolmogorov–Smirnov tests and all three data sets were found to be normally distributed (Kolmogorov–Smirnov, $p > 0.05$) for NDE subjects and not normally distributed (Kolmogorov–Smirnov, $p < 0.05$) for DE subjects. The mean \pm SD was used to describe the results from NDE subjects, while the median \pm IQR was used to describe the results for DE subjects.

Spearman's correlation was used to investigate the relationship between all data obtained from the three tests (PRT, McMonnies and TF). Pearson's correlation was used to study the relationship between the tests in NDE subjects, while Spearman's correlation was used in DE subjects. Correlation coefficients were graded as: small (0.10–0.29), medium (0.30–0.49), and large (0.50–1.00) [40].

Mann–Whitney test was used to study the differences between the NDE and DE groups in TF, PRT and McMonnies based on the TF scores.

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