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

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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 ± 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±4.3mm and 0.78±0.40, respectively. Median McMonnies, PRT and TF grade in DE subjects were 16.5±3.0, 9.0±2.0mm and 2.3±1.48, respectively. In NDE subjects, grades 0.0 to 1.8 were observed (82.5% Grade 0.0 to 1.00). Grades 2.0 to 4.0 were observed in DE subjects (72.5% Grades 2.0 to 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.

Keywords: Tear ferning; dry eye disease; Masmali grading scale; phenol red thread test

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 epithlelia, 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 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 (1999, 2007) 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 5-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 5 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 ± 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 ⅓ 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 seconds 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 (1µl) were collected from the lower meniscus of the right eye using glass capillary tubes (10µl, Drummond Scientific Company, USA) and allowed to dry on a clean, unused glass slide for 10 minutes under normal room temperature (23ºC) and humidity (40%). Samples were immediately observed under digital microscope (Olympus DP72) with 10X magnification. Each ferning pattern observed was graded using the 5-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 to 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 3 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 ± SD was used to describe the results from NDE subjects, while the median ± 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 to 0.29), medium (0.30 to 0.49), and large (0.50 to 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.

3. Results

The mean and median values for McMonnies questionnaire, phenol red thread test, and tear ferning grading scale are shown in Table 1.

Table 1. The mean ± SD (NDE) and median & IQR (DE) for McMonnies questionnaire scores, PRT test and TF grading scale

Test	Mean ± SD	Median (IQR)
	NDE	DE
McMonnies questionnaire	7.1±3.8	16.5 (15.0–18.0)
PRT (OD)	27.4±4.3	9.0 (8.0–9.0)
TF grading scale (OD)	0.8±0.4	2.3 (2.10–3.60)

For the NDE eyes, TF grades from 0.0 to 1.8 were observed, with the majority (82.5%) of samples within the range of 0.0 to 1.0. For the DE subjects, grades from 2.0 to 3.0 were observed in the majority (72.5%) of samples, with the remaining 27.5% of subjects having TF grading scale range of 3.1 to 4.0. The frequency of grading for NDE and DE subjects is recorded in Tables 2 and 3, respectively. Samples of NDE and DE tear ferning images are shown in Figures 1 and 2, respectively.

Table 2. The frequency of grading for NDE subjects on the TF grading scale

TF grading scale	Frequency	Percentage (%)
0.0–0.5	11	27.5
0.6–1.0	22	55
1.1–1.5	4	10
1.6–1.8	3	7.5

Table 3. The frequency of grading for DE subjects on the TF grading scale

TF grading scale	Frequency	Percentage (%)
2.0–2.5	23	57.5
2.6–3.0	6	15
3.1–3.5	1	2.5
3.6–4.0	10	25

Figures 1–2 here

In NDE subjects (Table 4), a medium, negative correlation was found between TF grade and PRT test, and between PRT test and McMonnies score. There was negligible correlation between the TF and McMonnies scores.

In DE subjects (Table 5), there was a small negative correlation between TF grade and PRT test; and a medium negative correlation between TF grade and McMonnies score. There was also a negligible correlation between PRT test and McMonnies score.

When all subject data (Table 6) was grouped together, there was a large negative correlation between PRT and McMonnies score (Figure 3), and between PRT and TF grade (Figure 4). There was also a large positive correlation between TF grade and McMonnies score (Figure 5).

Table 4. Correlations between TF grade, PRT and McMonnies score in NDE subjects

		TF	PRT	McMonnies
TF	Pearson Correlation	1.00	–0.30	0.03
	Sig. (2-tailed)		0.07	0.84
	N	40	40	40
PRT	Pearson Correlation	–0.30	1.00	–0.32
	Sig. (2-tailed)	0.07		0.04
	N	40	40	40
McMonnies	Pearson Correlation	0.03	–0.32	1.00
	Sig. (2-tailed)	0.84	0.04	
	N	40	40	40

Table 5. Correlations between TF grade, PRT and McMonnies score in DE subjects

		TF	PRT	McMonnies
TF	Spearman's Correlation	1.00	–0.20	–0.30
	Sig. (2-tailed)		0.22	0.06
	N	40	40	40
PRT	Spearman's Correlation	–0.20	1.00	0.12
	Sig. (2-tailed)	0.22		0.47
	N	40	40	40
McMonnies	Spearman's Correlation	–0.30	0.12	1.00
	Sig. (2-tailed)	0.06	0.47	
	N	40	40	40

Table 6. Correlation between TF grade, PRT and McMonnies score in all subjects' data

		TF	PRT	McMonnies
TF	Spearman's Correlation	1.00	–0.79	0.73
	Sig. (2-tailed)		0.00	0.00
	N	80	80	80
PRT	Spearman's Correlation	–0.79	1.00	–0.60
	Sig. (2-tailed)	0.00		0.00
	N	80	80	80
McMonnies	Spearman's Correlation	0.73	–0.60	1.00
	Sig. (2-tailed)	0.00	0.00	
	N	80	80	80

Figures 3–5 here

For further analysis, all of the subjects were first of all divided into four groups based on the TF scores (Group I: 0–1; Group II: 1.1–2; Group III: 2.1–3; Group IV: 3.1–4) and secondly into two groups based on TF scores (NDE subjects: 0–1.8; DE subjects: 2–4). This second classification was based on a TF grade of 1.8, which was found to be the maximum TF grade in NDE subjects, while a grade of 2 was the minimum for DE subjects. The data for all subjects was not normally distributed and non-parametric tests were used.

There was a significant difference within the four groups (Kruskal-Wallis; $p < 0.01$) and within the two groups (Kruskal-Wallis; $p < 0.01$) for TF, PRT and McMonnies. A Mann-Whitney test found a significant difference between the NDE and DE groups in TF grade ($z = -7.71$; $p < 0.01$), PRT ($z = -6.39$; $p < 0.01$) and McMonnies score ($z = -7.70$; $p < 0.01$), based on the TF grade.

4. Discussion

The 5-point Masmali grading scale [31] was introduced to provide a reliable method of tear fern grading that would overcome the limitations within Rolando's grading scale, especially the lack of sensitivity in categorisation of ferning patterns due to the overlap across Type I & II grades. In a previous published study, the 5-point scale was found to be discriminating, linear and reliable [31].

This study has applied the 5-point grading scale on tear ferning patterns obtained from NDE and DE subjects, using 0.1 increments, to examine the validity of the scale to differentiate between these subject types. The use of 0.1 increments has been suggested to increase test sensitivity [39]. In NDE subjects, 82.5% were found to have a grade between 0.0 and 1.0, with the remaining 17.5% to have grades from 1.1 to 1.8. In sharp contrast, 72.5% of DE ferning patterns were graded between 2.1 to 3.0, and 27.5% graded from 3.1 to 4.0. There was no overlap in TF grading for this group of subjects, as classified using the McMonnies questionnaire.

Statistical analysis of the results found a significant difference within the TF grades between NDE and DE subjects. When the subjects were classified into either two or four groups, based on the tear ferning scores, there was always a significant difference in the PRT and McMonnies score between the groups. This reverse analysis of the data indicates that the tear ferning test, based on the new grading scale, has the ability and sensitivity to differentiate between NDE and DE subjects.

Taking the full data set for all subjects, the tear ferning grading scores had a large correlation with both the PRT and McMonnies questionnaire results. A medium correlation was found between TF grade and PRT in NDE subjects and between TF grade and McMonnies scores in DE subjects. The moderate strength of these correlations may help in explaining the poor relationship noted between ocular signs and symptoms in dry eye [41]. If dry eye is often, but not always, associated with a deficient aqueous component volume [2], then changes in the content of the aqueous component may be detectable with tear ferning. This hypothesis is supported by the Chi-square test, which showed very strong evidence for a relationship between tear ferning, PRT and McMonnies. While a previous study [42] reported little relationship between TF score and other tear film tests, this may be due to limitations in the grading scale used. In contrast, the use of the new tear ferning grading scale in this study has allowed a stronger correlation with some tests to be identified. All of these results support the role of tear ferning as a useful diagnostic test, with the potential to work well with other diagnostic tests.

Based on the statistical analysis, we propose that a tear ferning pattern Grade ≥ 2 can be taken as a cut-off grade between non-dry eye and abnormal, with any ferning pattern less than Grade 2 considered as representing a non-dry eye tear film. This classification will help support practitioners to grade and evaluate the ocular tear ferning patterns.

Tear ferning test is a simple and inexpensive test, which has features that make it suitable for application in the eye clinic when evaluating the tear film [31], and the new 5-point tear fern grading scale has the ability to distinguish between non-dry eye and dry eye subjects.

Further application of the tear ferning test with the Masmali grading scale is needed to support the role of this test in the clinic and to help in the diagnosis and management of dry eye disease. The next useful step is to compare the chemical analysis of the collected tear sample with its tear ferning pattern, matching it with the grading scale.

5. Conclusions

A tear ferning Grade ≥ 2 can be classified as representing an abnormal pattern. The Masmali 5-point TF grading scale has shown good validity. The tear ferning test can be used as a clinical and research method to detect the dryness of the eye and investigate the tear film along with other additional tests.

Disclosure

None of the authors has any proprietary interest in this manuscript.

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Figures Captions

Figure 1. Sample of tear ferning pattern obtained from a NDE subject (equivalent to Grade 0).

Figure 2. Sample of tear ferning pattern obtained from a DE subject (equivalent to Grade 3).

Figure 3. Correlation between PRT test and McMonnies score for all subjects.

Figure 4. Correlation between tear ferning grade and PRT test for all subjects.

Figure 5. Correlation between tear ferning grade and McMonnies score for all subjects.

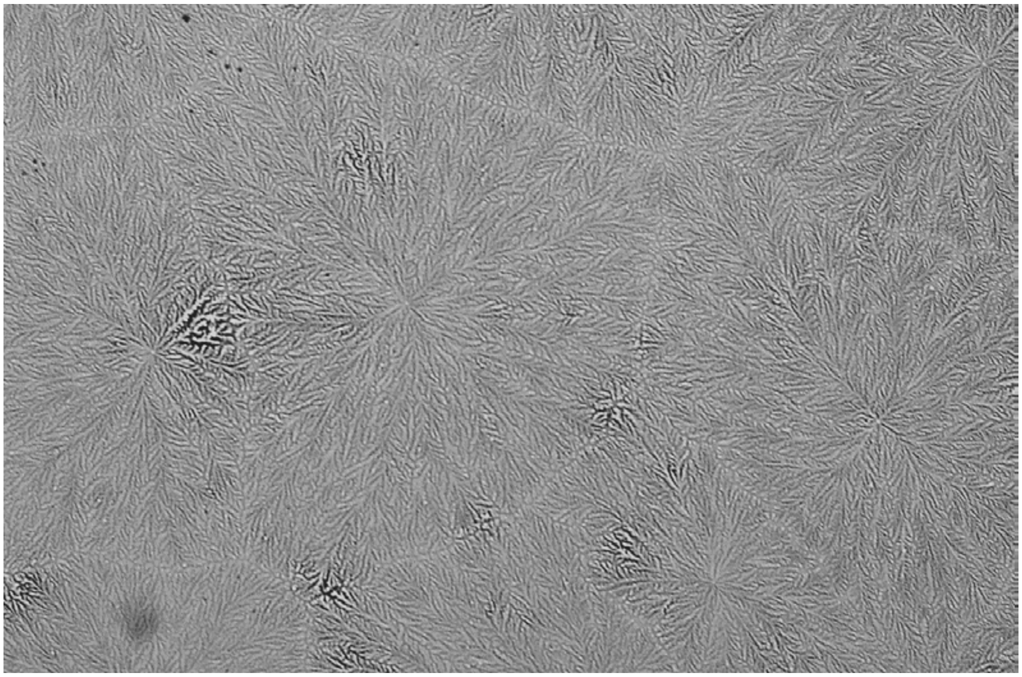

Figure 1. Sample of tear ferning pattern obtained from a healthy eye subject (equivalent to Grade 0).

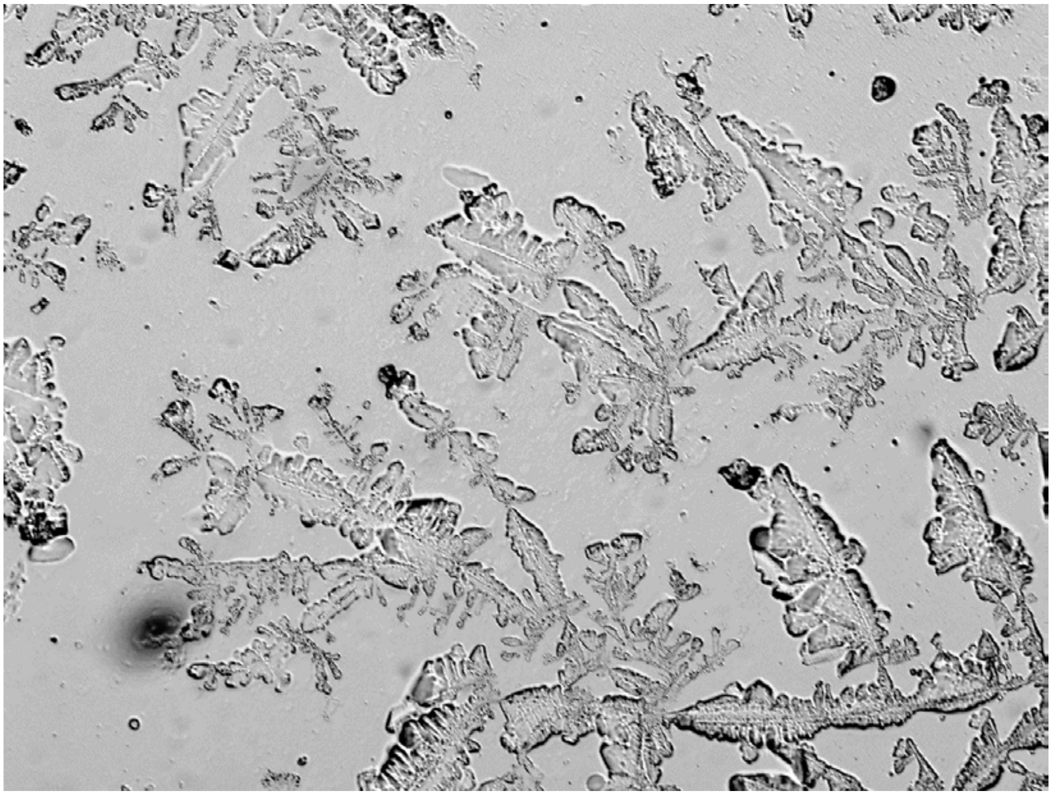

Figure 2. Sample of tear ferning pattern obtained from a dry eye subject (equivalent to Grade 3).

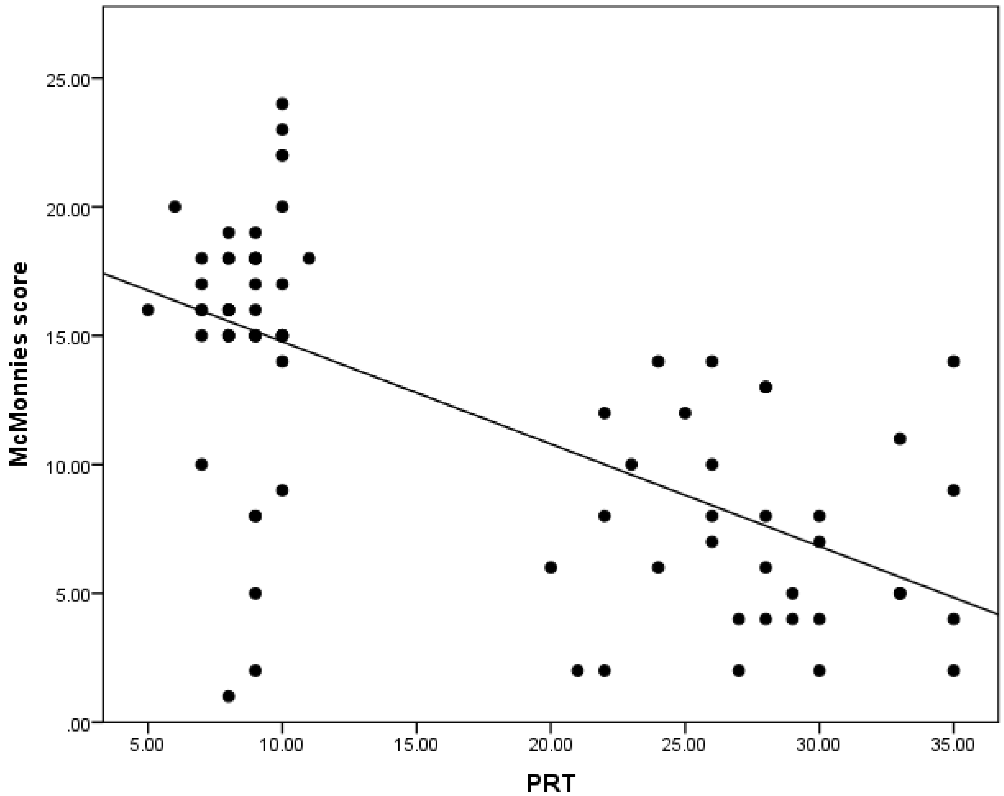

Figure 3. Correlation between PRT test and McMonnies score for all subjects.

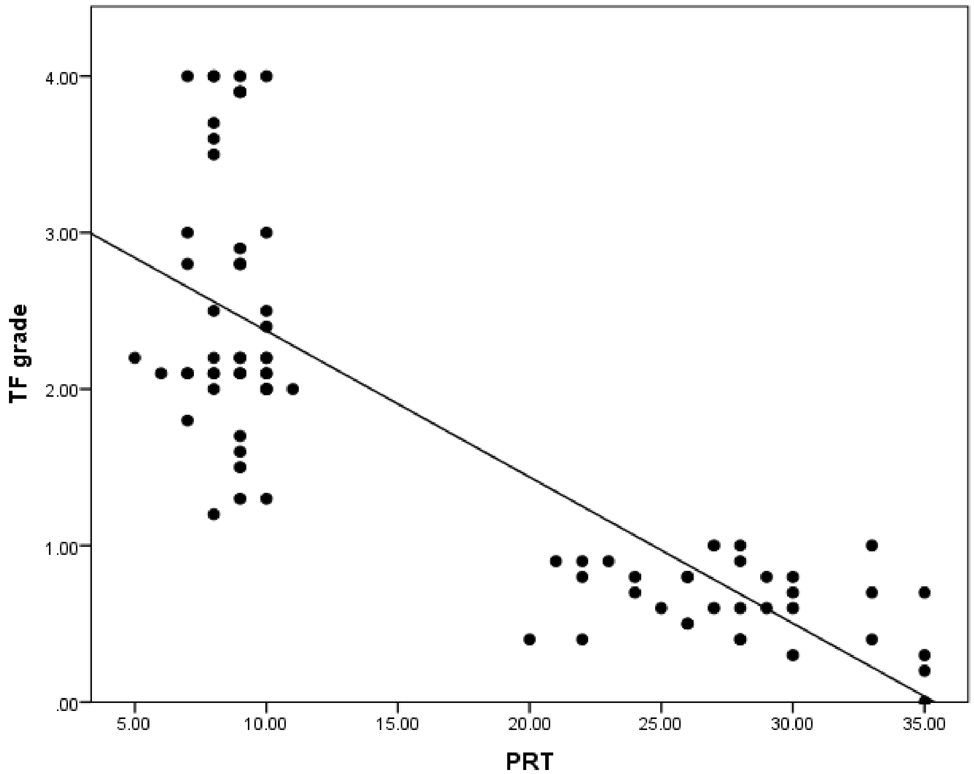

Figure 4. Correlation between tear ferning grade and PRT test for all subjects.

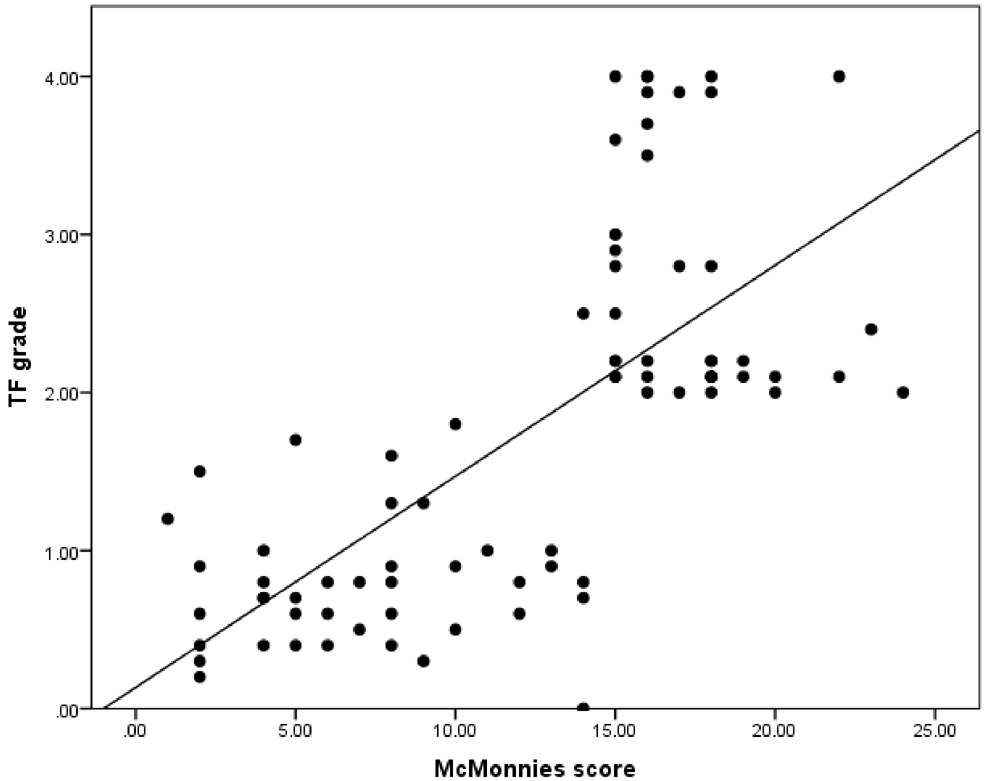

Figure 5. Correlation between tear ferning grade and McMonnies score for all subjects.