The Impact of Diurnal Changes and Inter-Visit Variability on the Concentration of Insulin-like Growth Factor-1 in Human Tears

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INTRODUCTION

There is a growing body of research focused on the use of tear film-derived proteins as non-invasive biomarkers of disease. Previous studies have reported quantitative changes in tear-derived growth factors and related proteins associated with various systemic and ocular diseases that may help monitor disease and response to therapy.[1,2] For tear film proteomic studies however, low abundant proteins such as growth factors are often masked by the presence of a small number of highly abundant proteins, which constitute more than 80% of the tear film proteome.[3] For more targeted studies of the low abundant tear film constituents, enzyme-linked immunosorbent assays (ELISA) that require substantial tear volumes are frequently used.

PURPOSE

The purpose of this study was to investigate the impact of diurnal changes and inter-visit variability on the concentration of a low abundant growth factor present in human tears, the insulin-like growth factor-1 (IGF-1), using a standard ELISA.

MATERIALS AND METHODS

Research Participants: Nine healthy non-contact lens wearers asymptomatic for dry eye were recruited in this study. All patients signed an informed consent.

Dry Eye Exam: All patient's underwent a standard dry exam at the initial visit. This included a brief biomicroscopic examination and assessment of corneal staining. Tear film stability was assessed by measuring the tear film break up time using fluorescein. Basal tear production was measured using a Schirmer's test with anesthesia. A diagnosis of dry eye was based upon the criteria outlined in the 2007 Dry Eye Workshop.[4]

Tear Collection: Six tear samples were collected for each patient (total of 54 clinical visits). Tears were collected at two time points (early-mid morning and late afternoon) on three different days to assess both diurnal fluctuation and inter-visit variability in tear IGF-1 concentration. At each visit, minimally stimulated basal tears were collected from the inferior temporal tear meniscus using glass microcapillary tubes. Four µl were collected from the right eye (for total protein determination) and 12µl were collected from the left eye (for IGF-1 concentration). The samples were stored at -80C until use.

Bicinchoninic Assay: Total protein was measured using a standard bicinchoninic assay.

ELISA: Tear concentration of IGF-1 was measured using a Human IGF-I Quantikine ELISA Kit (R&D Systems).

RESULTS

Table 1: Patient demographics and dry eye assessments

	Total (n=9)	NDE (n=4)	CSDE (n=5)	P value
Age (Mean ± SD) Range	35.7 ± 7.4 years (range 26-51)	40.5 ± 7.9 years (range 32-51)	31.8 ± 4.4 years (range 26-38)	p = 0.073
Gender	M = 2, F = 7	M = 1, F = 3	M = 1, F = 4	p = 1.000
Schirmer's Scores (Mean ± SD)	20.7 ± 13.0 mm	32.9 ± 4.3 mm	13.1 ± 7.5 mm*	p = 0.001
TFBUT (Mean ± SD)	7.4 ± 4.6 sec	11.0 ± 4.9 sec	4.5 ± 0.9 sec	p = 0.022

NDE: no dry eye; CSDE: clinical signs of dry eye
*One patient in the CSDE group had a normal Schirmer's score (23 mm) with reduced TFBUT (4.6 sec). The remaining 4 patients in the CSDE group had Schirmer's scores <11 mm and TFBUT <6 sec.

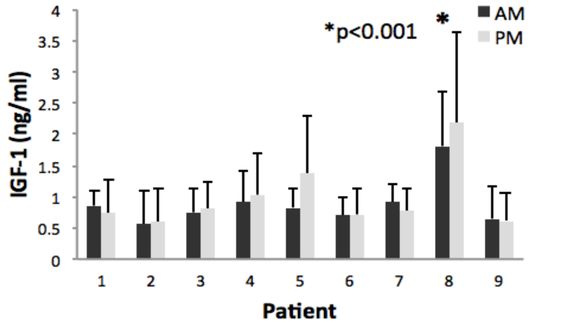


Figure 1: Diurnal variation in IGF-1 and total protein levels . (A) There was no significant difference in tear IGF-1 values between samples collected in the morning and the afternoon (p=0.236, two-way ANOVA, SNK multiple comparison post hoc). One participant (patient 8), had significantly high levels of IGF-1 compared to all other patients (*p<0.001). This patient was difficult to collect tears from and demonstrated a reduced tear volume. (B) There was no difference in total protein between morning and afternoon (two-way ANOVA, p = 0.123, patient; p = 0.639, time). Data expressed as mean ± standard deviation.

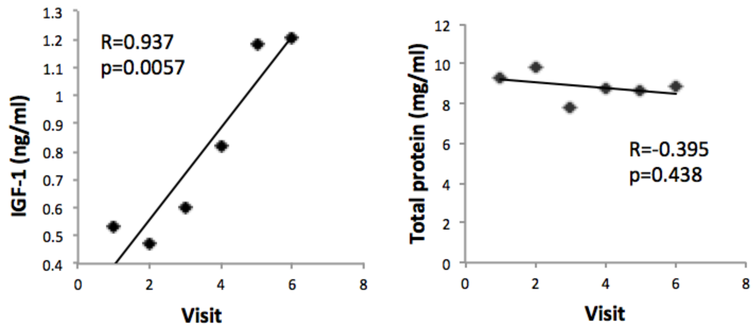


Figure 3: Inter-visit variation in total protein levels. (A) There was a significant correlation between visit number and tear IGF-1 concentration over the 6 visits (Pearson's correlation coefficient, R=0.937, p=0.0057). (B) In contrast to this, total protein levels were unchanged (Pearson's correlation coefficient, R= -0.395, p = 0.438).

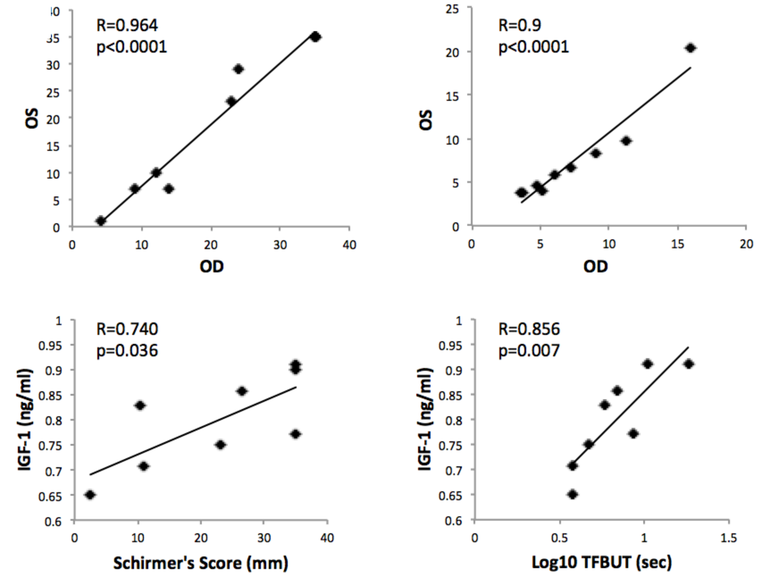
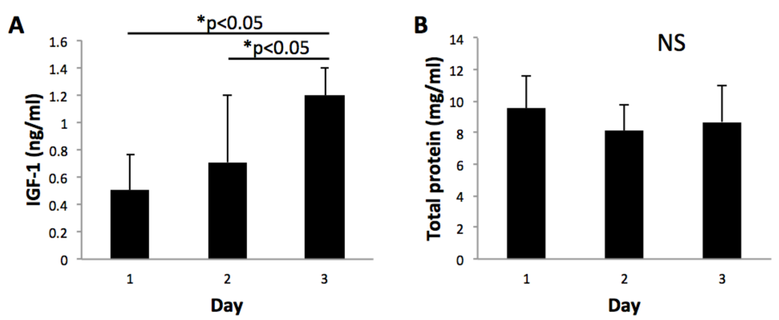


Figure 4: Relationship between tear IGF-1 and dry eye status. (A-B): For each individual patient, there was a high correlation between left eye and right eye values obtained for Schirmer's Score (A, R=0.964, p<0.0001, Pearson's) and TFBUT (B, R=0.9, p<0.0001, Pearson's). (C-D) Tear levels of IGF-1 were correlated with Schirmer's Score (C, R=0.740, p=0.036) and with TFBUT (D, R=0.856, p=0.007, Pearson's). Total protein level was not affected by tear production (R=0.2354, p=0.577), nor tear stability (R=0.047, p=0.911), data not shown. Due to the significantly high level of IGF-1 in tears collected from patient 8 (Fig. 1A), patient 8 was not included in this analysis.

- Neither tear levels of IGF-1 nor total protein was affected by time of tear collection (Fig. 1).
- Mean IGF-1 levels were significantly increased with each subsequent visit over baseline (time point 1). By visit 6, there was a two-fold increase (p<0.05) over baseline (Fig. 2).
- In contrast to IGF-1, total protein was unchanged at each visit (Fig. 2).
- With the exception of patient 8, IGF-1 concentration was correlated with dry eye status with patients exhibiting mild dry eye signs demonstrating an increase in IGF-1 (p<0.05). This effect was not evident with total protein (p=0.223, Fig 3).

Figure 2 (right): Day to day variation in tear IGF-1 concentration. (A) IGF-1 levels were significantly increased in tears collected on day 3 (RM one-way ANOVA, p<0.05, SNK post hoc multiple comparison test). (B) There was no change in total protein levels on any of the days evaluated (RM, one-way ANOVA, p=0.223).



DISCUSSION

The results from this study demonstrate the impact of small changes in reflex tearing on the measurement of low abundant proteins, such as IGF-1, in human tear fluid; and identified two key factors that introduce variability into the measurement. The first is the level of participant conditioning to the tear collection method. As patient comfort level with the technique increased, so did the examiner's ability to collect a sample containing minimally stimulated (basal) tears. Second, the presence of any mild dry eye signs also influenced IGF-1 concentration. Since the eye commonly "waters" in response to dryness, the potential increase in reflex tearing resulted in a corresponding reduction in tear IGF-1. The exception to this was patient 8 who presented with a low tear volume, making it difficult to collect the tear sample. The mean concentration of IGF-1 on day 3 (1.2 ng/ml) is in agreement with our previous reports of tear IGF-1 levels in a much older patient cohort where normal reflex tearing would be reduced due to aging.[5] With the increasing use of tear film-derived biomarkers in disease, the establishment of reliable parameters for obtaining accurate and repeatable values for concentrations of both high and low abundant tear proteins is required.

REFERENCES

1. Zhou et al. *Prog Retin Eye Res* 2012.
2. Von Thun Und Hohenstein-Blaul et al. *Exp Eye Res* 2013.
3. Zhou et al. *J of Proteomics* 2012.
4. Report of the 2007 International Dry Eye WorkShop (DEWS). *Ocular Surface* 2007.
5. Wu et al. *Ocular Surface* 2012.

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