Growth Hormone & IGF Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Growth Hormone & IGF Research

journal homepage: www.elsevier.com/locate/ghir

Shifting the IGF-axis: An age-related decline in human tear IGF-1 correlates with clinical signs of dry eye

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ARTICLE INFO

Keywords:
Tears
IGF-1
Dry eye
Aging

ABSTRACT

Objective: The human corneal epithelium expresses both the insulin-like growth factor type 1 receptor (IGF-1R) and the IGF-1R/insulin receptor (INSR) hybrid. Despite the previous identification of IGF-1 in human tear fluid, little is known regarding the regulation of IGF-1 in tear fluid and its role in corneal epithelial homeostasis. In the present study, we investigated the impact of biological parameters on the concentration of human tear levels of IGF-1.

Design: Tear levels of IGF-1 were measured in 41 healthy, human volunteers without any reported symptoms of dry eye. All volunteers underwent standard biomicroscopic examination of the cornea and tear film. In a subgroup of volunteers, corneal staining with sodium fluorescein, tear film break up time and tear production using a Schirmer's test strip were measured to assess clinical signs of dry eye. Tears were collected from the inferior tear meniscus using glass microcapillary tubes and IGF-1 levels were measured using a solid phase sandwich ELISA.

Results: Tear levels of IGF-1 were highest in young adults and significantly decreased in older adults ($P = 0.003$). There were no differences in tear IGF-1 between males and females ($P = 0.628$). Tear IGF-1 levels were correlated with tear film break up time ($R = 0.738$) and tear production ($R = 0.826$).

Conclusions: These data indicate that there is a progressive decline in tear IGF-1 due to aging that is associated with clinical signs of dry eye. This effect is likely due to age-related changes in the lacrimal gland.

1. Introduction

Dry eye disease is thought to affect millions of adults in the United States with an estimated prevalence ranging from 5 to 50% [34]. Severe dry eye is associated with significant morbidity and negatively impacts quality of life. Common risk factors for dry eye disease include systemic disease, female sex, and advanced age; however, multiple factors including medications, contact lens wear and ocular surgery can also contribute to dry eye [23,24,30,34,36]. For a complete review on risk factors for dry eye disease, the reader is directed to two recent reviews on pathogenicity and iatrogenic causes of dry eye [3,11]. Dry eye disease can be classified as aqueous deficient dry eye defined by insufficient tear production from the lacrimal gland, and evaporative dry eye, due to alterations in the lipid composition of the tear film. At the proteomic level, the healthy tear film is composed of a few high abundant tear proteins, including lysozyme, lactoferrin and immunoglobulin, which exist in mg/ml concentrations [9,31,37,41]. These highly abundant proteins constitute > 80% of the tear film proteome [10]. This is followed by moderately abundant proteins, which approximate concentrations in the μg/ml range and are easily identified through mass spectrometry. Finally, low abundant proteins range from ng/ml to pg/ml concentrations and are often masked by more abundant proteins, requiring sensitive assays such as enzyme linked immunoassays (ELISAs) for individual identification and quantification.

Insulin-like growth factor-1 (IGF-1) is a pleiotropic growth factor with known roles in proliferation, migration and survival of epithelial cells [5,17,45,46]. While both the IGF-1 receptor (IGF-1R) and the IGF-1R/insulin receptor (INSR) hybrid (Hybrid-R) are abundantly expressed in the corneal epithelium, the role of IGF-1 in maintaining normal corneal epithelial homeostasis is unclear [26,32,45]. Our *in vitro* studies indicate that IGF-1, and not insulin, activate IGF-1R and Hybrid-R to regulate cell proliferation. In a prior study, we reported the presence of IGF-1 and its principal binding protein, IGF-binding protein 3 (IGFBP-3), in healthy and diabetic human tears [44]. We further showed that the increased ratio between IGFBP-3 and IGF-1 in diabetic tears is sufficient to inhibit IGF-1 activation of the IGF-1R.

Studies characterizing changes in the IGF-1 axis indicate that measured levels of IGF-1 in serum are influenced by both age and sex

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https://doi.org/10.1016/j.ghir.2018.02.001
Received 5 December 2017; Received in revised form 22 January 2018; Accepted 4 February 2018

[12,14,39]. The impact of biological variables on tear concentration of IGF-1 is unknown. Given the multitude of factors that can affect measurement of low abundant growth factors in tears, the purpose of this study was to extend our prior findings and investigate the impact of parameters such as age, sex, and dry eye on tear concentration of IGF-1, a low abundant growth factor present in tear fluid.

2. Materials and methods

This study conformed to the tenets outlined in the Declaration of Helsinki. All procedures were approved by the Institutional Review Board at the University of Texas Southwestern Medical Center and all patients signed an informed consent prior to participating in the study. A total of 41 patients were recruited for this study. For inclusion, all patients were non-contact lens wearers without any ocular disease or any history of ocular surgery. All patients denied having a diagnosis or any symptoms or complaints of dry eye and none required the use of artificial tears or any other therapy for dry eye. Five microliter of tears were collected from the right and then left eye. For this single visit study, all tears were collected between 8 and 10 AM. All volunteers underwent tear collection as the first clinical test. Visualization of a normal tear meniscus height was required for tear collection and inclusion in the study. After allowing sufficient time for tear film recovery, subjects completed a slit lamp examination of the cornea and tear film, including basic dry eye measures of tear film break up time, tear production and corneal staining, as detailed below.

2.1. Tear collection

Using a Haag-Streit slit lamp biomicroscope with low illumination, minimally stimulated basal tear fluid was collected from the inferior temporal tear meniscus using 1 or 2 µl glass microcapillary tubes (Sigma, St. Louis, MO), with care to avoid touching the eye to minimize reflex tearing. A total of 10 µl were collected and pooled from both eyes. Samples were immediately placed on ice and stored at −80 °C until use.

2.2. Assessment of cornea and tear film

Health of the cornea and ocular surface was assessed by a single clinical examiner (DMR). Following a brief biomicroscopic examination to rule out any existing corneal pathology, the ocular surface was evaluated for corneal staining using fluorescein, and measures of tear film break up time (TFBUT) and basal tear production were obtained. For the determination of tear film break up time, 2 µl of 2.0% non-preserved fluorescein (Greenpark Pharmacy, Houston, TX) were instilled onto the superior bulbar conjunctiva. After 3 normal blinks, the duration between the last blink and the first dark spot was timed using a stopwatch. A total of 3 readings were recorded with 30 s rest periods in between and values were averaged. Immediately following, the cornea was evaluated for staining. Corneal staining was graded using a modified-NEI approach that consisted of assessment of corneal staining in five regions using a scale of 0–3 in 0.1 increments [1,18,29]. For all fluorescein-based measurements, a Wratten #12 filter was used. As the last clinical test, basal tear production was assessed using a Schirmer's tear test 1 with anesthesia [20,42]. The Schirmer's strip was placed in the lower fornix near the lateral canthus and the subject was instructed to close their eyes. The length of the wetted area after 5 min was measured [20]. All tests were performed for both eyes and the values for the right and left eyes were averaged to achieve a final measurement.

2.3. ELISA

Tear levels of IGF-1 were determined from pooled samples collected from the right and left eyes of each patient using a human IGF-I Quantikine ELISA Kit (R&D Systems, Minneapolis, MN), a solid phase sandwich ELISA. For analysis of each sample, 10 µl of tears were diluted in assay buffer to bring the resulting sample volume up to 50 µl. The ELISA was performed according to manufacturer instructions. Final concentrations were measured using a Bio-Rad iMark microplate absorbance reader (Bio-Rad, Hercules, CA) at 540 nm with wavelength correction and calculated from a standard curve of known concentrations of human recombinant IGF-1.

2.4. Bicinchoninic assay

In the subgroup of patients undergoing dry eye testing, an additional 6 µl was collected to allow for measurement of total tear protein. Total protein was measured using a bicinchoninic assay (BCA, ThermoFisher Scientific, Richardson, TX) according to manufacturer instructions. Concentration was measured using a Bio-Rad iMark microplate absorbance reader at 562 nm.

2.5. Statistical analysis

Statistical analysis was performed using Sigma Plot 11.0 (Systat Software, Inc., San Jose, CA). All data are expressed as mean ± standard deviation. A One-way ANOVA was used to test for differences in tear IGF-1 between age ranges. A Mann-Whitney Rank Sum test was used to assess differences in tear IGF-1 levels between sexes. A Pearson's correlation coefficient was used to test for correlations between variables including tear IGF-1 level and age and between tear IGF-1 and clinical measures of dry eye. A Shapiro-Wilk test was done to test for a normal distribution in age and dry eye measures. A log transformation was performed to achieve a normal distribution for TFBUT. Statistical significance was set at $P < 0.05$.

3. Results

As summarized in Table 1, in the total cohort, 22 patients were female and 19 were male. The mean age was 45.8 ± 10.8 years. Mean tear IGF-1 concentration was 1.16 ± 0.36 ng/ml. When stratified by age, tear IGF-1 was highest in participants aged 20–29 (2.02 ± 0.10 ng/ml) and decreased with each successive decade (Fig. 1). Tear IGF-1 was significantly decreased by 50% in participants aged 50–59 and remained decreased for patients aged 60–69 ($P = 0.003$, One-way ANOVA, Dunn's *post hoc* multiple comparisons test). Correlation analysis showed a strong negative correlation between age and tear IGF-1 concentration ($R = -0.756$, $P = 0.00009$, Pearson's correlation coefficient). There was no significant difference in tear IGF-1 concentration between females and males, 1.14 ± 0.29 ng/ml and 1.18 ± 0.43, respectively ($P = 0.628$, Mann-Whitney Rank Sum Test, Fig. 2).

In the subgroup tested for dry eye, 7 patients were female and 2 were male. The mean age in this smaller cohort was 35.7 ± 7.4 years. Mean tear protein concentration in this group was 8.8 ± 2.1 mg/ml, which is consistent with prior reports. None of the patients presented with clinically significant staining (defined as > 3.0/15.0 or 20% using the NEI industry scale). The mean Schirmer's score for subjects was normal (20.7 ± 13.0 mm) and the mean TFBUT time was 7.4 ± 4.6 s.

Table 1
Patient demographics.

	Age	Sex
Study cohort	45.8 ± 10.8 years (range: 25–66)	M = 19 (46.3%) F = 22 (53.7%)
Subset with ocular surface testing	35.7 ± 7.4 years (range: 26–51)	M = 2 (22.2%) F = 7 (77.8%)