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Inflammatory proteins associated with contact lens-related dry eye

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ABSTRACT

Purpose: To evaluate the levels and regulation of tear film inflammatory proteins in contact lens-related dry eye (CLDE).

Methods: One hundred healthy, daily wear (non-overnight), experienced soft contact lens wearers were classified into normal (n = 50) and CLDE (n = 50) groups based on Contact Lens and Dry Eye Questionnaire scores, tear break-up times, and comfort (a two-hour difference between total and comfortable daily lens wear hours). Tear samples (up to 5 μL) were collected by capillary extraction from the inferior meniscus of each eye, and pooled tear samples (10 per group) were tested using a customized Quantibody array. Mann Whitney tests with the Benjamini-Hochberg procedure with a 5% false discovery rate were used to compare the normal and CLDE groups.

Results: Relative to the normal group, the CLDE group showed a significantly increased tear concentration of several inflammatory mediators, including interleukin (IL)-7 ($p = 0.001$), IL-8 ($p = 0.001$), IL-13 ($p = 0.001$), IL-15 ($p = 0.001$), IL-12 p70 ($p = 0.002$), growth-related oncogene-alpha/ chemokine (C—X—C motif) ligand 1 ($p = 0.003$), granulocyte–colony stimulating factor ($p = 0.005$), IL-11 ($p = 0.008$), epidermal growth factor receptor ($p = 0.01$), IL-1 receptor antagonist (RA) ($p = 0.013$), macrophage colony-stimulating factor ($p = 0.013$), Eotaxin/C—C motif chemokine ligand 11 (CCL11) ($p = 0.016$), and IL-2 ($p = 0.016$). The following cytokines were increased three-fold or more in the CLDE group: IL-13 ($p = 0.001$), Eotaxin/CCL11 ($p = 0.016$), and IL-1RA ($p = 0.013$).

Conclusions: Several inflammatory markers, including interleukins, were increased in tears of subjects with CLDE. These results support a growing body of evidence that suggests a potential role of inflammation in CLDE.

1. Introduction

A healthy and stable preocular tear film is a prerequisite for the maintenance of ocular surface health. Destabilization of the tear film due to external or local factors upsets the delicate homeostatic balance at the ocular surface and gives rise to disorders, including dry eye disease (DED)—a common ocular condition associated with serious quality of life consequences and affecting millions worldwide [1,2]. In DED, excessive evaporation of tears causes instability and hyperosmolarity of the tear film, producing symptoms of discomfort. The resultant tear hyperosmolarity directly or indirectly initiates a vicious cycle of epithelial stress and desiccation, inflammatory events, and ocular surface damage [3]. Although evaporation-induced hyperosmolarity plays a significant role in driving this cascade of events, other factors may still initiate the DED cycle and perpetuate the disease [3].

Contact lens-wear is a significant risk factor that may provide a point of entry to the vicious cycle of DED. Studies have shown that the risk of developing DED increases by 2 to 3-fold due to contact lens wear [4–7]. When placed on the eye, the presence of a contact lens separates the precorneal tear film (PCTF) into pre-lens and post-lens compartments, producing several biophysical and biochemical alterations in the PCTF [8]. For example, the interaction of contact lens with the PCTF results in an increased rate of evaporation, reduced tear thickness and volume, delayed spreading of the lipid layer, reduced PH, increased tear ferning, increased osmolarity of the pre-lens tear film, and increased friction between the lens and surface epithelium, all of which can predispose to DED and can cause symptoms of discomfort [9,10]. Ocular discomfort due to dryness has been identified as a primary reason for the discontinuation of the lens [11–13]. Even among the current lens wearers, over 50 % report symptoms of dry eyes and may have contact lens-related dry

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eye (CLDE) [14]. It has been shown that contact lens wearers are 12 times more likely to experience symptoms of discomfort and dryness than emmetropes who do not wear lenses [14]. Apart from ocular discomfort, CLDE can also have functional consequences, including decreased visual performance and lens wear time, and an increased risk of infection [11,15,16].

It is now well established that inflammation is a core mechanism underlying the pathological process of DED and subsequent ocular surface damage. In DED, hyperosmolarity-induced epithelial stress initiates mitogen-activated protein kinase and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling pathways. The activation of these pathways stimulates interleukin-1 (IL-1) and tissue necrosis factor-alpha (TNF-α) production, which amplifies inflammatory response through the activation of several other mediators and cell signals and imparts damage to the corneal epithelial barrier through upregulation of matrix metalloproteinase-9 (MMP-9) [3]. Several inflammatory mediators are known to be present in the tear film [17], and studies have consistently shown DED is associated with upregulation of these mediators, including interleukins IL-1α, IL-1β, IL-6 and IL-8, TNF-α and MMP-9 [3,18–21], supporting an etiological role of inflammation in DED. Many of these markers have also been found to be increased in normal contact lens wearers compared to normal non-lens wearers [9,22–26], raising a possibility that contact lens wear *per se* may be intrinsically inflammatory [27].

The role of inflammation in contact lens-induced dry eye (CLDE) and ocular discomfort, however, remains unclear [28,29]. While some have found no difference in the concentration of 11 tear cytokines, including IL-1β, IL-6, and IL-8, between symptomatic and asymptomatic contact lens wearers [30], others have shown correlations between ocular comfort and the concentration of vascular endothelial growth factor (VEGF) [31], prolactin-induced protein [32], leukotriene B_4 (LTB_4) [33], with the latter being significantly higher in symptomatic than non-symptomatic contact lens wearers [34]. Higher levels of transforming growth factor-beta (TGFβ1), neurokine nerve growth factor (NGF) and immune response markers, such as human leukocyte antigen (HLA-DR) and CD23 have also been reported in tears of symptomatic lens wearers [35,36]. Given the significant involvement of inflammatory events in DED pathogenesis and the overlap of symptoms of ocular discomfort and tear film characteristics between DED and CLDE, it seems logical that inflammation plays a role in CLDE. This study was designed to investigate a wide range of inflammatory markers, including interleukins, which are known to be associated with DED, using a rigorously defined CLDE. It was hypothesized that subjects with CLDE with clinically measurable tear film instability will show an increase in the concentration of several tear proteins, including the interleukins, associated with the inflammatory processes.

2. Materials and methods

2.1. Subjects

Ethical approval for the study was obtained from the Institutional Review Board prior to recruitment. All subjects were treated in accordance with the tenets of the Declaration of Helsinki. Out of 181 interested subjects who were initially phone-screened to verify age and type and duration of lens wear (see inclusion criteria below), 132 passed the telephone screening and were asked to visit the research clinic to determine further eligibility for participation in the study. Written informed consent was obtained from all subjects prior to enrollment. However, of those who were enrolled and attended the study visit, 32 did not meet all three classification criteria (described below) and were excluded. The remaining 100 subjects completed the study visit and were included in the analysis.

All subjects were required to be healthy, daily soft contact lens wearers with at least one-year experience of lens wear. Other inclusion criteria were age (18–39 years), lens wear for at least six hours per day and five days per week, and best-corrected visual acuity of at least 20/30 in each eye. Subjects using artificial tears and lubricants were included if they were willing to discontinue these medications on the day of the study visit. Exclusion criteria included overnight contact lens wear, daily disposable contact lens usage, active ocular disease (including glaucoma) and history of ocular infection, inflammation or allergy within the past 6 months, corneal refractive surgery or other ocular surgery that may affect the ocular surface, use of ocular medications containing active pharmaceutical agents affecting the ocular surface (including steroids, topical azithromycin, glaucoma medications, etc.), pregnancy, lactation, and systemic disease or medications that may affect the eye (e.g., Sjogren's syndrome, diabetes, rheumatoid arthritis, infections, hay fever). Use of nutraceuticals, such as omega-3 fatty acid supplements, was acceptable if it was not initiated or altered within two weeks prior to the study visit.

Eligible subjects were classified into normal and CLDE groups based on three criteria: Contact Lens and Dry Eye Questionnaire (CLDEQ) scores [37], difference between total and comfortable daily lens wear hours (normal $<$ 2 hours, CLDE $\geq$ 2 hours), and tear break up time (TBUT, normal $\geq$ 7 seconds, CLDE $<$ 7 seconds, described below). Subjects had to fulfill all three criteria to be classified into either group.

2.2. Tear sample collection

Tear film samples were collected, with care to avoid reflex stimulation, from the inferior-temporal tear meniscus in both eyes, by an experienced examiner (PR), with the contact lenses in situ. Subjects were instructed to look in the opposite direction during sample collection. Up to 5 μL of basal tear sample from each eye was collected by capillary extraction and frozen at −80 °C until lab analysis. During collection, contact was made with only the tear meniscus; care was taken to avoid any contact with the lid margin/ ocular surface, as such a contact may stimulate reflex tearing. If the initial pooled tear sample from both eyes was less than three μl, additional tears were collected after a minimum of 20 min rest since the initial tear collection. The total tear volume included sample volume from both eyes and collection sessions as applicable.

2.3. TBUT assessment

Following removal of contact lenses, subjects were given a break to ensure stabilization of the tear film. Approximately five minutes after contact lens removal, five μl of liquid fluorescein (0.5 %) was then pipetted on the ocular surface, and subjects were asked to blink three times to ensure adequate mixing of fluorescein in the tear film. The tear break-up time was measured in each eye as the time interval between a complete blink and appearance of a dark spot (break) in the tear film, under cobalt blue illumination setting of the slit lamp and using a yellow Wratten filter. Three measurements were recorded, and an average was calculated for each eye.

2.4. Tear protein quantitation

Tear protein concentration was assessed using Bradford assays. This procedure included the addition of 250 μl of Bradford working reagent (Sigma-Aldrich Co., St. Louis, MO/ USA) to two μl of each sample, followed by the measurement of the optical density at 595 nm using a TECAN Infinite M200 reader (TECAN Group Ltd., Männedorf / Switzerland). Corresponding protein concentrations were then derived from a standard curve developed using bovine serum albumin (Sigma-Aldrich Co., St. Louis, MO/ USA). Subsequently, total protein from each sample was calculated based on tear protein concentrations and respective tear volumes.

2.5. Cytokine array analysis

A customized quantitative antibody array (Quantibody® Human Cytokine Antibody Array, RayBiotech Inc., Norcross, GA, USA) was used to perform an assay of inflammatory mediators in the tear film. The array panel consisted of 50 commonly observed inflammatory proteins, including cytokines and chemokines (see Tables 1 and 2). Each array slide consisted of 16 wells, eight wells for standards and eight for study samples, and each well contained 50 cytokine antibodies printed in quadruplicate. An equal amount of protein (15 μg) per sample pooled from tears of 5 subjects each containing 3 μg protein was used for the array analysis (subject pooling strategy detailed below), and the arrays were treated per the manufacturer's protocol described below. Test cytokines and sample quantity to be loaded were derived from optimization experiments reported previously wherein the array's sensitivity, selectivity and linearity of the signal were assessed using tear samples from normal and dry eye subjects [38].

2.6. Pooling strategy

In each group (normal/CLDE), tear samples were sorted based on average TBUT values (mean of two eyes). Tear samples (containing 3 μg tear protein per subject) within each group were then pooled from groups of subjects (sorted by average TBUT values, see Supplementary Table 1) for testing using the array slides. A total of 20 pooled samples (10 normal/ 10 CLDE) were eventually tested. Because the tear samples were pooled, the average would be most representative for subject grouping rather than the individual TBUT values from each eye. Using average TBUT is a common practice in both patient care and clinical trials, as it increases the precision of the estimate, and is recognized as the standard operating diagnostic procedure [39].

Table 1
Frequency Distribution of Contact Lens and Care Solution in the Normal and CLDE Groups.

		Frequency (%)		Chi-square statistic	p-value
		CLDE group	Normal group		
FDA group	1	37 (74)	39 (78)	0.42	0.936
	2	2 (4)	1 (2)		
	3	2 (4)	2 (4)		
	4	6 (12)	7 (14)		
Silicone Hydrogel	0	8 (16)	10 (20)	0.18	0.671
	1	39 (78)	39 (78)		
Modality	Biweekly	33 (66)	31 (62)	0.17	0.677
	Monthly	17 (34)	19 (38)		
Material	Senofilcon A	21 (42)	18 (36)	13.3	0.653
	Lotrafilcon A	4 (8)	7 (14)		
	Galyfilcon A	5 (10)	4 (8)		
	Etafilcon A	3 (6)	5 (10)		
	Comflcon A	3 (6)	5 (10)		
	Lotrafilcon B	3 (6)	1 (2)		
	Balafilcon A	2 (2)	2 (2)		
	Enfilcon A	1 (2)	2 (4)		
	Omafilcon A	2 (4)	1 (2)		
	Methafilcon A	0 (0)	2 (4)		
	Ocufilcon D	1 (2)	1 (2)		
	Polymacon	0 (0)	2 (4)		
	Phemfilcon A	0 (0)	1 (2)		
	Tetrafilcon A	0 (0)	0 (0)		
Care solution (coded by preservative)	Hydrogen peroxide	17 (34)	13 (26)	2.19	0.701
	Polyquad	12 (24)	15 (30)		
	Generic PHMB	13 (26)	10 (20)		
	Branded PHMB	8 (16)	12 (24)		

CLDE: Contact lens-related dry eye.
Note: A few subjects did not provide adequate information during the survey. These were coded as missing or incomplete data and excluded from this Table.

Table 2
Inflammatory Proteins Found in Significantly High Concentration in Contact Lens-Related Dry Eye (CLDE) in Comparison to Normal Subjects.

Inflammatory protein	Normal Mean ± SD (pg/mL)	CLDE Mean ± SD (pg/mL)	p-value *
IL-7	5.8 ± 2.4	10.7 ± 4.7	0.001
IL-8	11.7 ± 5.1	22.4 ± 8.7	0.001
IL-13	0.5 ± 0.5	2.1 ± 1.4	0.001
IL-15	26.4 ± 12.1	48.7 ± 15.6	0.001
IL-12 p70	3.1 ± 1.8	6.0 ± 2.0	0.002
GRO-α/ CXCL1	454.4 ± 234.3	919.5 ± 350.9	0.003
GCSF	4.7 ± 2.8	11.1 ± 5.6	0.005
IL-11	8.4 ± 4.0	13.9 ± 3.9	0.008
EGFR	44.4 ± 23.0	69.3 ± 22.4	0.010
IL-1RA	229.1 ± 212.8	734.7 ± 632.7	0.013
MCSF	10.6 ± 4.0	16.1 ± 3.8	0.013
EOTAXIN/ CCL11	261.9 ± 129.8	896.5 ± 447.5	0.016
IL-2	18.0 ± 7.3	24.9 ± 5.3	0.016
HB-EGF	20.9 ± 18.6	55.4 ± 34.7	0.02†
IFNγ	84.7 ± 52.5	131.4 ± 28.4	0.03†
IL-5	50.4 ± 36.4	63.7 ± 11.9	0.03†
IL-6	3.7 ± 2.9	6.4 ± 2.5	0.03†
IL-12 p40	29.0 ± 26.1	62.4 ± 33.7	0.04†

CLDE: Contact lens-related dry eye.
* p-values from Mann-Whitney U tests.
† Not significant after Benjamini-Hochberg correction for multiple comparisons with false discovery rate at 0.05.

2.7. Antibody array analysis

Reconstituted cytokine standards were serially diluted to make eight concentrations used for generating the standard curve for each analyte, and 100 μL of standard cytokines was applied to the array wells. Individual tear samples containing 15 μg tear proteins were diluted to 100 μL with sample diluent and applied to the wells. The slide was incubated at 4 °C overnight with gentle shaking. Following the recommended wash regimen, 80 μL of detection antibody cocktail was added to each well and incubated at room temperature for three hours. Slides were washed again, and 80 μL of Cy3 equivalent dye-conjugated streptavidin was added to each well, before covering the glass chip with aluminum foil and incubating at 4 °C overnight. Slides were washed and dried by centrifuging at 1000 rpm for minutes and then imaged using GenePix 4000B Microarray scanner (Molecular Devices, Sunnyvale, CA, USA) with a pixel resolution of 5 μm set at 532 nm and photomultiplier tube 600. Finally, pixel data were extracted from the images using the array specific. gal file provided by the manufacturer.

2.8. Data analysis

Data were analyzed using the Quantibody® Q-analyzer from RayBiotech. Sample cytokine concentrations were calculated in pg/mL from linear regression functions derived from the standard curves. All standard curves were revised to eliminate outlier data points including signal saturation to improve the linearity of the regression model. Cytokine concentrations with signal intensities lower than the standard curve range were labeled as below limits of detection. Statistical analysis was performed using SPSS software (IBM Corp., Armonk, N.Y., USA). Two sample t-tests were used to compare age, CL wear durations, and overall tear volume and protein concentration between the two groups, while the chi-square test was used to compare categorical variables. Differences in cytokine concentration between the two groups were tested using Mann Whitney tests, followed by Benjamini-Hochberg procedure with a false discovery rate of 5% (5% of features identified significant are truly null) to control for possible false positives due to multiple testing. Data are presented as mean ± SD unless stated otherwise.

3. Results

3.1. Subject demographics and classification parameters

Of the total 100 subjects who completed the study, 50 subjects were classified as normal and 50 as having CLDE. The average age of the subjects was 23.7 ± 3.7 in the normal group and 25.9 ± 4.9 years in the CLDE group (t = -2.59, p = 0.01). The two groups were well matched for sex, with 52 % of the normal group and 68 % of the CLDE group being female ($\chi 2 = 2.67$, p = 0.10). The average CLDEQ score was 0.28 ± 0.31 in the normal group and 1.56 ± 0.66 in the CLDE group. The CLDE group had on average 4.15 ± 1.04 s tear breakup time, while the normal group had 8.02 ± 1.03 s.

The two study groups had a similar duration of contact lens wear history, averaging about 10 total years of contact lens wear (normal: 10.0 ± 3.9 years, CLDE: 10.4 ± 4.5 years, p = 0.57). As expected, the normal group demonstrated significantly longer comfortable contact lens wear time (13.4 ± 2.6 h vs. 8.1 ± 3.29 h, p < 0.0001) and total contact lens wear time (13.8 ± 2.5 h vs. 12.3 ± 3.0 h, p = 0.01) than the CLDE group. There was no difference in the type of CL or CL care solution used between the normal and CLDE groups (Table 1). Details on contact lens wear duration (including days and hours of lens wear), type of CL and lens care used, and the effects of these parameters on the dry eye status of these subjects have been published elsewhere [40].

3.2. Overall tear volumes and protein concentrations

The average total tear volume collected from the normal subjects (7.12 ± 2.10 μL) was significantly higher than the CLDE subjects (5.90 ± 1.84 μL, p = 0.002). The total tear protein demonstrated a similar trend, with a significantly higher total tear protein in the normal group (32.49 ± 15.89 μg) compared with the CLDE group (26.22 ± 14.30 μg, p = 0.04). However, there was no difference in tear protein concentration between the normal group (4.59 ± 1.65 μg/μl) and the CLDE group (4.23 ± 1.65 μg/μl, p = 0.28).

3.3. Array analysis

Table 2 provides a list of the cytokines that were significantly higher in the CLDE group compared with the normal group, while Table 3 summarizes the concentrations of the remainder of the cytokines tested. Relative to the normal group, the CLDE group showed a significantly increased tear concentration of several inflammatory mediators, including IL-7 (p = 0.001), IL-8 (p = 0.001), IL-13 (p = 0.001), IL-15 (p = 0.001), IL-12 p70 (p = 0.002), growth-related oncogene-alpha/ chemokine (C—X—C motif) ligand 1 (GRO-α/CXCL1) (p = 0.003), granulocyte–colony stimulating factor (GCSF) (p = 0.005), IL-11 (p = 0.008), epidermal growth factor receptor (EGFR) (p = 0.01), IL-1 receptor antagonist (RA) (p = 0.013), macrophage colony-stimulating factor (MCSF) (p = 0.013), Eotaxin/C—C motif chemokine ligand 11 (CCL11) (p = 0.016), and IL-2 (p = 0.016). The following cytokines were increased three-fold or more in the CLDE group: IL-13 (p = 0.001), Eotaxin/CCL11 (p = 0.016), and IL-1 receptor antagonist (RA) (p = 0.013) (Fig. 1). The concentrations of five other cytokines (Heparin-binding EGF-like growth factor (HB-EGF), interferon (IFN)-γ, IL-5, IL-6, and IL-12 p40) were not different between the normal and CLDE groups after controlling for the false discovery rate (Table 2). Some of the tested cytokines were below limits of detection. These included granulocyte-macrophage colony-stimulating factor (GM-CSF), I-309, IL-1α, IL-1β, IL-4, IL-6sR, macrophage inflammatory protein (MIP)-1d, MMP-13, RANTES, sTNF-RI, sTNF-RII and TNFβ.

Table 3
Summary Statistics of Other Tested Cytokines in Contact Lens-Related Dry Eye (CLDE) and Normal Groups.

Inflammatory protein	Normal Mean ± SD (pg/mL)	CLDE Mean ± SD (pg/mL)	p-value *
EGF	228.8 ± 134.3	335.9 ± 94.6	0.06
MIP-1α/ CCL3	568.8 ± 421.2	824.5 ± 307.2	0.06
BLC/ CXCL13	4.9 ± 2.3	7.5 ± 3.1	0.07
Eotaxin-2/ CCL24	0.9 ± 0.8	2.0 ± 1.4	0.08
IL-16	7.2 ± 4.8	10.6 ± 3.2	0.11
IL-17	10.8 ± 7.5	16.8 ± 7.7	0.11
MIP-1β/ CCL4	2.1 ± 1.4	3.4 ± 2.0	0.11
TNFα	6.3 ± 9.4	7.8 ± 5.3	0.13
MMP-2	3391.9 ± 1995.8	4026.1 ± 1226.5	0.17
ICAM-1	601.9 ± 413.8	971.4 ± 563.6	0.20
MMP-3	163.2 ± 91.6	195.4 ± 68.2	0.23
TIMP-2	458.6 ± 194.7	539.0 ± 129.4	0.23
VEGF	108.6 ± 26.8	119.5 ± 28.1	0.45
MMP-1	28.9 ± 12.6	23.9 ± 9.6	0.50
MMP-9	307.2 ± 343.6	450.1 ± 488.7	0.50
PDGF-BB	2.8 ± 1.9	2.3 ± 0.8	0.65
MCP-1/ CCL2	103.3 ± 75.6	122.9 ± 104.6	0.71
MIG/ CXCL9	62.8 ± 85.5	37.7 ± 16.1	0.71
TIMP-1	12644.0 ± 5553.0	13640.4 ± 7088.4	0.76
IL-10	16.9 ± 8.4	17.7 ± 8.5	0.94

CLDE: Contact lens-related dry eye.
* p-values from Mann-Whitney U tests.

4. Discussion

It is generally accepted that inflammation plays a critical role in the pathogenesis of DED. However, the role of inflammation in CLDE and associated discomfort remains inconclusive. The objective of this study was to compare the concentrations of 50 inflammatory mediators in tears of contact lens wearers classified as normal subjects and subjects with CLDE based on symptoms (CLDEQ scores), tear film instability (TBUT), and comfort (the difference between total and comfortable daily lens wear hours). Of the 50 proteins tested, 38 were detected in tears of contact lens wearers. Out of these 38 inflammatory mediators, 13 were found to be significantly upregulated in CLDE subjects compared with the normal subjects. This finding suggests that inflammation may be involved in the etiology of contact lens-related dry eye and discomfort commonly experienced by contact lens wearers.

4.1. Inflammation in contact lens wear

Tears contain a wide range of inflammatory mediators, including cytokines, chemokines, adaptive and immune cells, and prostaglandins [17]. Several studies have now shown that contact lens wear itself predisposes individuals to elevated tear concentrations of these inflammatory mediators. In a group of neophyte contact lens wearers, Thakur and Willcox reported decreased levels of IL-8, LTB_4, and IL-6 but higher numbers of polymorphonuclear leukocytes (PMNs) in tears after 8 h of sleep in comparison with a non-contact lens wearing group [23]. Pisella et al. showed upregulation of HLA-DR and intercellular adhesion molecule type 1 (ICAM-1) in asymptomatic soft contact lens wearers compared with the non-lens wearers [22]. Schultz and Kunert detected IL-6 in contact lens wearers but not in non-lens wearers; the concentration of IL-6 in lens wearers decreased to non-detectable levels six days after discontinuation of lens wear and returned to their original levels after 24 h of resuming lens wear [24]. Dionne et al. demonstrated upregulation of eotaxin-2, GM-CSF, I-309, and monocyte chemoattractant protein–1 but downregulation of IFN-g, IL-2, IL-6, IL-6 soluble receptor (IL-6sR), IL-7, IL-8, IL12p40, IL-13, monocyte-colony stimulating factor, macrophage inflammatory protein–1a, TNFβ, TNF receptor I (sTNF-RI) and II contact lens wearers when compared with non-contact lens wearers [41]. Differential expression of inflammatory mediators does not appear to be limited to daily wear modality, as an increased concentration of epidermal growth factor has also been reported in subjects wearing silicone hydrogel lenses for an extended period [25, 42]. Markoulli et al. showed that once overnight wear of the contact lens significantly increased the level of MMP-9, which returned to the

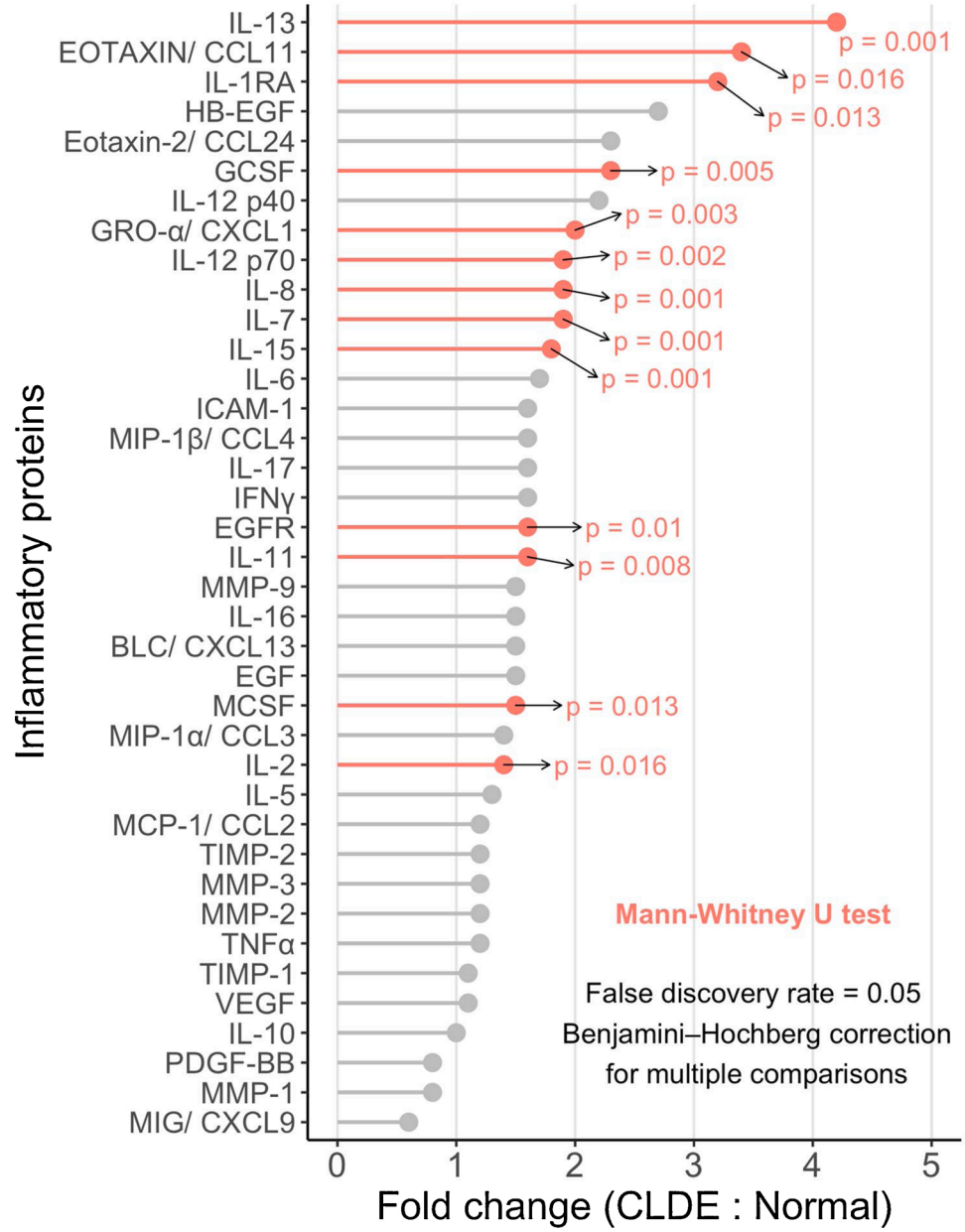

Fig. 1. Relative tear concentrations of various inflammatory proteins in subjects with contact lens-related dry eye (CLDE) compared with normal subjects. Relative concentration is expressed as the ratio of concentration in CLDE to normal subjects.

baseline by one month, suggesting a possible adaptive component in the inflammatory response [43]. While this study did not compare the concentration of inflammatory mediators between contact lens wearers and non-contact lens wearers, it found a large array of mediators in tears of daily soft contact lens wearers with and without CLDE. These findings are consistent with the growing notion that contact lens wear may itself be intrinsically inflammatory and can lead to a subclinical inflammatory state.

4.2. *Inflammatory proteins in CLDE*

Previous reports on the regulation of inflammatory markers in CLDE and contact lens discomfort show inconsistent results. Lopez-de la Rosa et al. studied 11 cytokines (EGF, fractalkine, IL-10, IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-8, MCP 1, TNF-α, and MMP 9) in tears of symptomatic and asymptomatic contact lens wearers but found no difference in the concentration of any protein [30]. By contrast, Liu and colleagues showed elevated NGF and TGFβ levels in contact lens wearers with dry eye compared with non-contact lens wearers [36]. Albeitz showed higher expression levels of HLA-DR and CD23 in subjects with CLDE [35]. Recent studies from Willcox and his team have provided further evidence that specific proinflammatory markers may undergo differential regulation in subjects experiencing symptoms of discomfort. Masoudi et al. reported that, among prostaglandins, leukotriene B4, cysteinyl leukotrienes, lactoferrin, lysozyme, lipocalin 1, proline-rich protein 4, and prolactin-induced protein, only the level of LTB_4 was significantly higher in symptomatic than asymptomatic contact lens wearers [34]. The authors also found an increased level of LTB_4 during the day as well as during lens wear, suggesting that inflammation could underlie the end of day discomfort frequently observed in lens wearers [33]. However, results from an earlier study from the same authors demonstrated that out of 15 cytokines tested, only change in VEGF concentration was correlated with comfort ratings [31]. Furthermore, this change in VEGF was more marked in non-lens wearers than lens-wearers [31]. A recent

study has reported that symptomatic contact lens wearers have higher levels of IL-17a than non-symptomatic lens wearers [44].

In contrast with these reports, several inflammatory mediators, including IL-7, IL-8, IL-13, IL-15, IL-12 p70, GRO-α/CXCL1, GCSF, IL-11, EGFR, IL-1RA, MCSF, EOTAXIN/CCL11, and IL-2 were found to be upregulated in subjects with CLDE compared with healthy contact lens wearers. Among these mediators, the concentration of cytokines such as IL-13, IL-1RA, chemokines such as EOTAXIN/CCL11 and GRO-α/CXCL1, and growth factor such as granulocyte-colony stimulating factor (GCSF) increased by two-fold or more in CLDE subjects. CLDE subjects also showed significantly increased levels of heparin-binding epidermal growth factor (HB-EGF), interferon-gamma (IFN-γ), and interleukins, such as IL-5, 6, 12 p40 in tears. However, they were not significant at a 5% false discovery rate, so the possibility of these five markers being false positives cannot be ruled out. Consistent with a previous report, this study also showed a higher total protein content in the normal subjects than subjects with CLDE [45].

Although these studies point toward a low-key inflammatory response in contact lens discomfort and CLDE, the reasons for the discrepancy in findings related to specific markers are difficult to ascertain but could be due to several reasons. First, most studies have used subjective instruments (comfort ratings by the subjects or symptom questionnaire) to investigate the regulation of inflammatory markers as it relates to contact lens-related discomfort and dry eye. In contrast, the present study used rigorously defined criteria based on a validated screening tool, tear film instability, and comfort classifying subjects with CLDE only if they fulfilled all three criteria. This approach is likely to capture true CLDE subjects, especially given the well-established disparity in the frequency and diagnosis of DED based only on signs or symptoms. Second, the number of inflammatory mediators tested in the previous studies are considerably lower than those investigated in the present study. Third, the regulation of the inflammatory proteins could vary according to the time course of the lens wear (e.g., acute vs chronic use). Fourth, the larger sample size of this cohort compared with the prior reports may have contributed to reducing the variability in the concentration of inflammatory proteins and may have led to the detection of differentially regulated markers on a larger scale. Fifth, methodological differences (e.g., variation in methods related to tear protein analysis) could have resulted in the inconsistency in findings. Studies have used a variety of methods for proteomic analysis of tears, including mass spectrometry, assays, and gel electrophoresis [31,34,36,45]. Finally, little consideration has been given to the absolute magnitude of changes in protein concentrations across the studies. The concentrations of several cytokines have been previously shown to undergo diurnal variation. While IL-8 has been reported to decrease during the day, other proteins like IL-7, IL-4, IL-12p70, and G-CSF have been reported to increase during the day [31]. However, these changes were modest, with only IL-8 showing nearly a two-fold change [31]. In contrast, most proteins that were found significantly different between the normal and CLDE groups in this study showed about two to four-fold change in concentration. Therefore, it is likely that changes in cytokine concentrations reported in this study are physiologically relevant to the tear film health.

Despite the discordance in findings among studies on CLDE and contact lens discomfort, these results are consistent with previous reports on inflammatory proteins in non-contact lens-related DED which show upregulation of several inflammatory markers, including EOTAXIN-1, IL-2, IL-5, IL-6, IFN- γ, IL-8 in both humans [18,46–48] and animals [49]. Some of these mediators like IL-5 and EOTAXIN-1 are known to be associated with ocular allergy and be increased in patients with allergies disorders including vernal keratoconjunctivitis, atopic keratoconjunctivitis and giant papillary conjunctivitis [50,51]. These findings support a potential involvement of allergy-mediated inflammatory pathways in CLDE as suggested previously [35]. Increased levels of stress responding chemokines like IL-6 and associated chemokines such as CCL11 support the involvement of both acute and chronic stress mechanisms in CLDE. The concentration of IL-6 in tears has been shown to correlate with tear film instability, tear production, epithelial desiccation and surface damage, and severity of dry eye symptoms [20]. The level of IL-8 in tears has also been shown to increase the frequency of corneal infiltrative events related to contact lens-associated inflammatory response [52]. While increased levels of IFN-γ in CLDE likely indicates T cell involvement, upregulation of anti-inflammatory factors like IL-1RA could be an attempt to suppress ocular surface inflammation as in DED [53,54].

4.3. Limitations of the study

Although this study has identified several inflammatory proteins that could be involved in the pathogenesis of CLDE-related inflammation, it has some limitations. While pooling of the samples within each group was necessitated by budgetary constraints, this approach may have diffused changes in concentration of very small magnitude across the samples. Although the classification of subjects into Normal and CLDE groups was based on three rigorously defined criteria, it is possible that the CLDE group may have represented both contact lens discomfort [8] and some elements of DED. In addition, the present study did not include a non-contact lens wearing group. Therefore, it is unable to characterize changes in protein concentrations induced by contact lens *per se* in healthy subjects. It is unclear as to why there were no changes in the concentration of prominent markers, including IL-1β, MMP-9, and other stress responders like IL-1 and TNF-α in the current study. Some possibilities include that these proteins remained unaltered in CLDE in adapted wearers, may have been onto the contact lenses (and depleted from the tear film), or may reflect a potential limitation of the array method used. Alternatively, regulation of these inflammatory markers may depend on the severity of CLDE, as it has been shown that the concentration of cytokines correlates significantly with the clinical severity of DED [53]. Inclusion of subjects with the more severe dry eye such as those with pronounced ocular surface staining may reveal further differences, although these patients may have most likely discontinued contact lens wear due to their disease state.

4.4. Conclusion

In conclusion, a variety of inflammatory markers including several interleukins were found to be upregulated in CLDE. This finding supports the potential involvement of inflammatory pathways relevant in CLDE. Further studies targeted at these inflammatory markers may provide deeper insights into the pathogenesis of CLDE and discomfort due to contact lens wear.

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Appendix A. Supplementary data

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