Cytokine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

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

Level of tear cytokines in population-level participants and correlation with clinical features

L. Tong*, T.Y. Wong, Y. Cheng

Yong Loo Lin School of Medicine, National University of Singapore, Singapore
Singapore National Eye Centre, Singapore
Singapore Eye Research Institute, Singapore
Duke-NUS Medical School, Singapore

ARTICLE INFO

Keywords:
Human study
Dry eye
Ocular surface inflammation
Tear protein
Cytokine
Population-based study

ABSTRACT

Aims: Tear cytokine levels indicate severity of ocular surface inflammation. Previous reports of cytokine concentrations were based on hospital-based studies or non-Chinese populations. We determine the range of tear concentration of cytokines in a representative adult Chinese population.

Methods: Thirty-nine participants were recruited from a population-based study of Chinese adults in Singapore, and standardized clinical ocular surface/eyelid features evaluated. Tear was extracted from Schirmer strips and analysed using a multiplex bead-based assay.

Results: Tear concentrations of 14 cytokines were investigated and quantifiable in each participant. Eight cytokines increased with increasing age, and 4 cytokines (IL-4, IL-12, IL-10 and IFN-γ) were increased in people with increased frequency of ocular discomfort. Three cytokines (MCP-1, IP-10 and IL-13) had increased levels in people with lower Schirmer tests, while 9 other cytokines were increased in patients with eyelid crusting (TNF-α, IL-1β, IL-17α, IL-2, IL-4, IL-6, IL-12, IL-10 and IFN-γ). Twelve percent of participants had eyelid crusting.

Conclusion: Using a convenient collection technique that is a routine clinical test, 14 tear cytokines could be quantifiable even in Singapore Chinese adults without a dry eye diagnosis. Elevation of different tear cytokines may be linked to subclinical aqueous tear deficiency or eyelid inflammation even in asymptomatic people.

1. Introduction

The profile of tear cytokines has been shown to be a *non-invasive biomarker* for ocular surface diseases [1]. Using *pooled* patient tear samples, inflammatory cytokines in the tears can be measured by ELISA [2]. However measurements for individual tear samples are preferred. Such assays are now possible, and can be multiplex for determination of multiple cytokines. They have been found to be reproducible, and a study that evaluated concentrations of 18 cytokines/chemokines in the tear of 24 young healthy adults found, with the exception of IL-10 and IL-1β, that there were no significant inter-day variations [3]. As tear production is routinely evaluated using a Schirmer I test, it is convenient to assess tear cytokines using this method of tear collection [4], and we further reported that miniaturization of this method using less reagents could be feasible [5].

Tear cytokine levels may shed light on the type of ocular surface disease. Tear cytokines have been shown to be raised in dry eye [6], and cytokines such as IL-17, TNF-α and IL-6 were further elevated in Sjogren's syndrome-related dry eye [7–9]. Aqueous tear deficiency, as compared to evaporative dry eye, may be associated with elevation of cytokines [10]. In conditions such as HIV infection, levels of cytokines in the tearfluid may also be disturbed [11]. Similarly, levels are altered in patients with diabetic retinopathy [12]. Currently, definitive therapy of dry eye is focused on reduction of ocular surface inflammation; changes in tear cytokines may therefore be monitored in patients to check treatment response [13].

However, hospital-based studies *inadvertently* include controls having a range of blepharitis or systemic conditions with unknown effects on the ocular surface [14]. Such controls do not represent the kind of individuals in general populations. It is useful to delineate the normal ranges of tear cytokines assayed using a specific technique, on participants that have not been referred to hospitals, in order to minimize confounding variables. The reference values should also be applicable to populations of specific ethnicity/demographics. The range of ten tear cytokines in a multiplex assay using tears eluted from Schirmer strips has been reported in America (5 subjects) [4] and in Spain (24 young healthy adults) [3]; seven tear cytokines have been reported in two studies of adult Koreans [7,15], and four tear cytokines using ELISA in

* Corresponding author at: Singapore National Eye Center, 11 Third Hospital Avenue, Singapore 168751, Singapore.
E-mail address: louis.tong.h.t@singhealth.com.sg (L. Tong).

https://doi.org/10.1016/j.cyto.2018.05.013
Received 22 February 2018; Received in revised form 14 May 2018; Accepted 15 May 2018

Table 1
Demographics and clinical data (n = 39).

Parameter	Summary
Female Gender	
Number (Proportion)	17 (43.6%)
Age	
Mean ± SD	58.2 ± 9.9
Median (Minimum, Maximum)	54 (49, 82)
Ocular surface signs	
Schirmer test (mm)	
Mean ± SD	8.9 ± 8.5
Median (Minimum, Maximum)	7 (0, 30)
Tear break up time (s)	
Mean ± SD	5.2 ± 2.9
Median (Minimum, Maximum)	4 (1, 16)
Corneal fluorescein staining	
Number (Proportion)	9 (23.1%)
Eyelid signs	
Presence of crusting on eyelids	
Number (Proportion)	5 (12.8%)
Presence of madarosis	
Number (Proportion)	3 (7.7%)
Presence of any sub-tarsal papillae or follicles	
Number (Proportion)	8 (20.5%)
Frequency of dry eye symptoms (graded 1–5)	
Dryness sensation	
Mean ± SD	1.6 ± 1.0
Median (Minimum, Maximum)	1.0 (1.0, 4.0)
Number (Proportion) with > 1	11 (28.2%)
Foreign body sensation	
Mean ± SD	1.2 ± 0.6
Median (Minimum, Maximum)	1.0 (1.0, 3.0)
Number (Proportion) with > 1	4 (10.3%)
Burning sensation	
Mean ± SD	1.0 ± 0.0
Median (Minimum, Maximum)	1.0 (1.0, 1.0)
Number (Proportion) with > 1	0 (0.0%)
Redness sensation	
Mean ± SD	1.5 ± 1.0
Median (Minimum, Maximum)	1.0 (1.0, 5.0)
Number (Proportion) with > 1	9 (23.1%)
Crusting of eyelids (sensation)	
Mean ± SD	1.1 ± 0.4
Median (Minimum, Maximum)	1.0 (1.0, 3.0)
Number (Proportion) with > 1	2 (5.1%)
Tendency to shut eyelids	
Mean ± SD	1.0 ± 0.0
Median (Minimum, Maximum)	1.0 (1.0, 1.0)
Number (Proportion) with > 1	0 (0.0%)

pooled samples from 270 Japanese people [2], but ranges of tear cytokine concentrations have not been reported in healthy Chinese populations.

We showed previously that lower Schirmer test values have been associated with raised tear concentrations in people with a known diagnosis of dry eye [16]. In the general population, high frequencies of people with abnormal Schirmers test and other dry eye tests, or have symptoms of dry eye are encountered [17], but these may not satisfy a formal diagnosis of dry eye, which would require the symptoms or signs to exceed thresholds or for these to be concurrently abnormal. In such populations, it is not clear if symptoms of dry eye, low Schirmer test readings, or signs of eyelid disease affect cytokine concentrations. Here, we aim to ascertain tear concentrations of 14 cytokines in an unbiased sample of adult Chinese participants from a population-level study, and investigate their potential associations with tear production, frequency of dry eye symptoms, and eyelid signs.

2. Methods

The sampling method of the Singapore Chinese Eye Study, a population cohort study, has been published elsewhere [18]. Briefly, age-stratified sampling of adults from 40 to 50 years, 50–60 years, 60–70 years and above 70 years were conducted based on residential status in South-western Singapore. All participants had given written informed consent. The study was approved by Singhealth centralized institutional review board. This report includes the results of tear collected from 39 adults which had been consecutively sampled from participants of the above cohorts who agreed to participate in an ocular surface sub-study. This study does not exclude any individuals, even those with abnormal eyelid signs. As more than half of un-referred individuals in a population could have meibomian gland dysfunction [19], the presence of individual eyelid signs may be part of the normal spectrum in healthy individuals.

The frequency of dry eye-related symptoms over the previous month was elicited by interviewers (with translation) as reported in the Salisbury Eye Evaluation Study (SEES) [20–22]. Schirmer I (without anesthesia), tear break up times and eyelid examination (for crusting, madarosis, dome formation, scalloping of lid margins and subtarsal papillary/follicular changes) were performed as described previously [23,24]. As this was not a clinical trial there is no exclusion criteria, and a comprehensive systemic and ocular examination were performed for all participants who came for the study.

The method of tear collection and 14-cytokine analysis using the bead-based multiplex indirect immunofluorescent (Bioplex) assay have been previously described [16]. Briefly the wetted portion of the Schirmer strip was excised and cut into tiny strips. The elution volume for each strip was 100 µL. The tear cytokine concentrations were normalized to 1 mm of Schirmer measurement (by dividing values by

Table 2
Tear cytokine concentrations in pg/mL (n = 39).

	Mean	SD	Median	Minimum	Maximum	2.5th percentile	97.5th percentile
IP-10	404.69	376.46	315.37	0.11	1694.40	0.11	1273.28
MCP-1	12.99	16.40	6.03	1.30	71.73	1.36	54.89
IL-8	12.36	26.60	4.88	0.50	164.30	0.50	43.52
IL-13	4.31	4.10	2.61	0.21	18.53	0.26	13.02
IL-4	3.68	6.21	1.14	0.00030	33.97	0.00054	14.87
MIP-1α	1.37	3.18	0.38	0.00032	14.35	0.00054	13.90
IFN-γ	1.06	1.37	0.62	0.00077	7.84	0.073	3.46
IL-10	0.77	1.49	0.36	0.00077	9.02	0.049	3.97
IL-6	0.73	1.44	0.40	0.00032	8.98	0.00075	2.49
IL-12	0.69	1.38	0.29	0.00032	8.20	0.00075	3.07
TNF-α	0.47	1.22	0.25	0.0069	7.80	0.031	1.27
IL-2	0.36	1.73	0.048	0.00067	10.86	0.00076	1.30
IL-1β	0.35	1.42	0.073	0.0046	8.94	0.013	1.29
IL-17A	0.29	1.45	0.0014	0.00030	9.02	0.00030	1.56