Cytokine 88 (2016) 77–84

Contents lists available at ScienceDirect

Cytokine

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

A distinct cytokines profile in tear film of dry eye disease (DED) patients with HIV infection

Rupesh Agrawal [a,b,*,1], Praveen Kumar Balne [a,1], Anuradha Veerappan [b], Veonice Bijin Au [c,d], Bernett Lee [c,d], Eileen Loo [a], Arkasubhra Ghosh [b,g], Louis Tong [b,e,f], Stephen C Teoh [h], John Connolly [c,d], Petrina Tan [i]

[a] National Healthcare Group Eye Institute, Tan Tock Seng Hospital, Singapore
[b] Singapore Eye Research Institute, Singapore
[c] Institute of Cell and Molecular Biology, Singapore
[d] Singapore National Eye Centre, Singapore
[e] Duke-NUS Medical School, Singapore
[f] National University of Singapore, Singapore
[g] GROW Research Laboratory, Narayana Nethralaya Foundation, India
[h] Eagle Eye Centre, Singapore
[i] Jurong General Hospital, Singapore

ARTICLE INFO

Article history:
Received 17 July 2016
Received in revised form 23 August 2016
Accepted 25 August 2016
Available online 29 August 2016

Keywords:
HIV
Dry eye
Tears
Cytokines
Inflammation
Luminex bead assay
Ocular surface

ABSTRACT

Purpose: To investigate the tear cytokine profile in HIV patients with dry eye disease (DED) and study the association between the severity of ocular inflammatory complications and tear cytokines levels. We postulate that HIV-mediated inflammation may be the underlying pathogenic mechanism for HIV-associated DED.

Methods: The current prospective case-control study compared tear film cytokine profiles in DED patients with HIV infection (n = 34) and age/gender-matched DED patients without HIV infection [controls (n = 32)]. Participants were recruited from tertiary referral eye care centre and communicable disease clinics, Singapore. Ocular surface health was documented using tear film, Schirmer's test, corneal staining, and conjunctival injection measurements. Tear samples were collected using Schirmer's strips and analysed for the levels of 41 cytokines using Luminex bead assay. Logistic regression models were performed to determine correlation and significance.

Results: Among the 41 cytokines analysed, statistically significant differences were observed in the mean values of epithelial growth factor (EGF), growth related oncogene (GRO) and interferon gamma-induced protein 10 (IP-10). EGF and IP-10 levels were higher and GRO levels were lower in the tears of DED patients with HIV infection compared to DED patients without HIV infection. No significant association was found between varying levels of ocular surface parameters and cytokine concentrations in HIV patients with DED ($p > 0.05$).

Conclusions: EGF and IP-10 were significantly elevated and GRO levels were lower in the tear profile of HIV patients with DED compared to immunocompetent patients with DED. This study suggests a novel cytokine driven paradigm for ocular inflammatory complications of HIV infection. Additional studies in large organised cohorts can validate the results.

1. Introduction

The eye is a common site for complications of human immunodeficiency virus (HIV) infection and several important HIV-associated disorders involve the anterior segment and ocular surface [1]. More than 50% of HIV-positive patients manifest anterior segment pathologies such as dry eye disease (DED), with 10–20% of patients having severe disease [2,3]. These numbers are greater than that in the general population [4], and affects HIV-infected children as well. However, the evaluation and management of these ocular surface disorders is overshadowed by more severe, blinding infections like cytomegalovirus retinitis. With improved treatment and survival of patients with HIV, the problem of ocular surface disease now demands increased attention [1].

* Corresponding author at: National Healthcare Group Eye Institute, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433, Singapore
E-mail address: rupesh_agrawal@ttsh.com.sg (R. Agrawal).
[1] Joint first authors.

http://dx.doi.org/10.1016/j.cyto.2016.08.026
1043-4666/

Numerous studies have been performed to investigate the pathogenesis of DED, especially those related to autoimmune diseases. However, no studies have been published to investigate the link between DED and HIV patients except for a study by Simbiri et al. demonstrating the dysregulation of cytokine expression in HIV patients with ocular surface squamous neoplasia [5]. The key gap in our current management is the lack of understanding of the pathogenetic mechanisms in HIV-associated ocular surface disease and DED; the most common ocular problem in these patients. It is also uncertain if its severity varies with the severity of HIV disease as reflected by blood parameters e.g. CD4 counts.

The current study aims to leverage on the important relationship linking the severity of ocular inflammatory diseases and infections with the array of cytokines and soluble proteins produced in tears. There is a compelling scientific argument that this relationship between tear cytokine levels and the severity of ocular surface disease extends to the study of HIV and AIDS, but this has never been explored.

In this case-controlled study, we postulate that HIV-mediated inflammation may be the underlying pathogenetic mechanism for HIV-associated DED. These mechanisms stimulate a cascade of inflammatory events and the generation of inflammatory cytokines from the glands or ocular surface. These cytokines are then reflected in the tears; as such, cytokine profiles in tear film of these HIV patients with DED would differ significantly from immunocompetent patients with DED. Our primary objective was to explore the cytokine profile in tear films of DED patients with HIV. Our secondary objective was to study the relationship between parameters of DED and tear cytokines levels.

2. Materials and methods

The current report is a prospective case-controlled study comparing cytokine profiles of tear films in HIV positive patients with DED (cases) versus immunocompetent patients with DED (controls). Institutional ethics board (National Healthcare Group, Domain Specific Research Board) approval was obtained and the study was conducted as per the Declaration of Tenets of Helsinki. Written informed consent was obtained from all the subjects after an explanation of the nature and possible consequences of the study. This study was approved by the institutional human experimentation committee or Institutional Review Board (IRB). We recruited 34 cases and 32 age/gender-matched disease controls and the exclusion criteria for the cases and controls was as follows:

- Subjects less than 21 years of age
- Subjects with concurrent ocular microbial infections on treatment (topical/intravitreal)
- Subjects diagnosed with other systemic diseases that affect tear production namely: autoimmune diseases (Rheumatoid arthritis, Sjogren's syndrome), thyroid disease, diabetes, connective tissue diseases
- Subjects with previous ocular surgeries/procedures e.g. LASIK
- Subjects who wear contact-lens
- Subjects who were pregnant
- Subjects with inability to comply with protocols indicated in this study or unable to give consent.

Cases were the HIV patients attending the eye clinic and the DED was diagnosed if participants complained of irritation, grittiness or foreign body sensation in last few weeks and if tear film break up time (TBUT) was less than 5 s. Immunocompetent patients with DED with similar clinical presentation as mentioned for HIV patients were included as controls.

2.1. Study procedure

Ocular surface health was evaluated in all the subjects by following investigations.

2.1.1. Assessment of ocular surface

Ocular examination included assessments of conjunctival injection, tear debris, filamentary keratitis, and presence of meibomian gland disease. Patients were graded on the severity of their dry eyes based on the grading scheme shown as per the guidelines proposed by International Dry Eye Workshop [6]. Tear collection was completed prior to any other testing. A 10-min interval was allowed between each test.

2.1.2. TBUT measurement

Five microliters of non-preserved 2% sodium fluorescein was instilled onto the bulbar conjunctiva without inducing reflex tearing by using a micropipette. The patient was instructed to blink normally without squeezing several times to distribute the fluorescein and then refrain from blinking until told otherwise. The slit-lamp magnification was set at 10×, and blue-light illumination was used to enhance observation of the tear film over the entire cornea. A stopwatch was used to record time between last complete blink and the first disruption of the tear film. After observing the TBUT, the patient was instructed to blink normally again. Three measurements were taken as recommended [7] and the average was recorded.

2.1.3. Corneal staining

The cornea was stained with 5 μl 2% Fluorescein solution and then examined under blue-light illumination for 2 min. The intensity of the corneal fluorescein staining was graded in each of four quadrants on the cornea (temporal, nasal, superior, and inferior) using a standardized four-point scale (0, no staining; 1, mild; 2, moderate; and 3, intense) (central zone was not recorded) [6].

2.1.4. Schirmer's test and collection of tear film

Schirmer's strips (Eye Care and Cure, Tucson, AZ, USA) were placed on the fornix of the patients' eyes for tear collection. After 5 min, the strip was removed from the eye, and the length of wetting was measured in millimeters. Then the strips were placed in microcentrifuge tubes and stored at −80 °C before eluting with Milliplex assay buffer (Merck, Billerica, MA, USA). The Schirmer's strips were incubated in 30 μl of assay buffer for 5 min at room temperature and centrifuged at 20,000 rpm for 1 min. The strips were discarded and the eluted tear samples were stored at −80 °C until cytokine analysis [8].

2.1.5. Clinical data collection

Clinical data of the patient's demographics and concomitant medical illness including laboratory results (including CD4/CD8 counts, and Full blood counts) and systemic treatment were collected at baseline. Patients who did not have recent blood work within 4 weeks of the study underwent updated blood lab testing.

2.1.6. Tear film cytokine analysis

The frozen tear extracts were thawed on ice, centrifuged at 3000 rpm for 5 min and 25 μl of each sample was used for the cytokine analysis. We estimated levels of 41 cytokines by Luminex bead-based multiplex assay using Milliplex® MAP human cytokine/chemokine magnetic bead panel −1 kit following the manufacturer's guidelines (Millipore, USA). The 41 analytes tested in each sample were epidermal growth factor (EGF), eotaxin, fibroblast growth factor (FGF)-2, Fms-like tyrosine kinase 3 ligand (Flt-3L), fractalkine, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF),