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# Utilization of High Level of Interleukin-10 in Tears as a Biomarker for Dry Eye Disease Detection in Computer Users

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**Dry Eye Disease (DED) can cause damage to the ocular surface, cornea, visual disturbances, and blindness. The causes of DED are multifactorial such as Video Display Terminals (VDT) activity with computers and electronic telecommunications equipment. The use of VDT for a long-time result in tears hyperosmolarity which directly affects the eye surface epithelium. This leads to an increase of inflammatory mediators, including interleukin-10 (IL-10), Interleukin-17, and other Cytokines. This study aims to prove that computer users for 4-hours/day with high IL-10 levels have a higher risk of DED than normal subject. Tear samples were collected from 46 volunteers which taken from Schirmer I examination, giving questionnaire to the subjects, and Tears Break Up Time (TBUT). The subjects then divided into case and control groups and levels of IL-10 were examined by ELISA test to detect Interleukin levels in tears. This study showed from all samples, 25 volunteers are DE and 21 healthy subjects with equal proportions between male and female. Chi-square analysis found the level of IL-10 were significantly increased in tears of DE patients. It showed that patients with high level of IL-10 has 4.3 times higher risk to get DED than patients in normal level of IL-10. The mean of TBUT test were significantly lower in DE patients.The mean of TBUT in case group was < 10 mm while in control group was > 10 mm, meanwhile the OSDI value were higher in case group than in the control group. The high levels of IL-10 in tears found as the risk factor of DED. It can become a potential biomarker for DED detection in computer users 4 hours/ day. It proves that inflammatory cytokines such as IL-10 have important roles in the immunopathogenesis of the DED.**

**Keywords:** Dry eye disease, Computer users, IL-10.

Dry eye disease (DED) is a global health problem and serious disorder of the tear film and can cause disorders in the ocular surface, cornea, visual disturbances, and blindness.[1] Dry eye syndrome is characterized by tear film instability and damage of the exposed surface epithelium.[1,2] The etiology of DED is multifactorial and many conditions have identified as independent risk factor of DED, one of them is Video Display Terminals (VDT). The experience visual symptoms often depend on the level ofthe amount of time spent looking at a digital screen such as computer, gadgets, electronic equipment.[3,4] The complex of eye and vision problems related to near work and

computer use called Computer Vision Syndrome (CVS).[5]

Based on the Tear Film and Ocular Surface Society (TFOS), Dry Eye Workshop II (DEWS II) 2015, the prevalence of DED ranged from 5-50% in world population[6]. The prevalence of DED with complaints without clinical symptoms is reported around 75%.[6]Dry eye syndrome causes significant decrease in quality of life and affects 3-34% of the adult population[7,8].The presence one of the 3 specific signs of homeostatic instability which includes a reduction in non-invasive break up time (NIBUT), increased tear osmolarity or the presence of ocular surface staining on the cornea, conjunctiva or lid margin represents a homeostatic instability that can confirm the diagnosis of DED[6].

Inflammation is an important factor in DED pathogenesis.[2,6]Dry eye disease due to long-term computer use decreases the blinking reflexes which will disrupt the tear layer and increasing the evaporation of aqueous layer in the tears film. In addition, computer electromagnetic radiation and ahigh temperature and low humidity environment will affect tear evaporation.[5,10,11]Computer-emitted blue rays contribute to the onset of photophobia and pain in the eye.[12,13]

Interleukin -10 (IL-10) is an immunomodulatory cytokine which plays role in T cells, dendritic cells and macrophages to reduce production of inflammatory cytokines and Antigen Presenting Cells (APC). [14]Recent research has shown that IL-10 activation during chronic inflammation is classic negative feedback mechanism, which initiates establishing a balance between activation and deactivation of cytokines at the site of inflammation[15]. Several studies have found there is an increasing of IL-10 levels in DED and ocular surface disease, such as in Sjogren's Syndrome (SS).[15,16]Meanwhile, study of IL-10 biomarkers in tears has never been reported in Indonesia.This study aims to prove that computer users for 4-hours/day with high IL-10 levels have a higher risk of DED than normal subject.

## METHOD

This is a case-control analytical study carried out 46 samples at Tax Office and Private Bank in Denpasar. The study was conducted from July - August 2018. Samples were finance staff at Private Bank and Denpasar Tax Office which collected by consecutive sampling after fulfilling the inclusion and exclusion criteria. The examination protocol was approved by the Institutional Ethics Committee of Faculty of Medicine, Udayana University, Denpasar.

The inclusion criteria for case group were: 25 – 35 years old subjects, works with computer 4 hours/ day, had symptoms of DED with OSDI score > 12.5 and TBUT < 10 seconds, willing to take part for the examination, and sign the informed consent. Inclusion for control group were 25 – 35 years old subjects, works with computer 4 hours/ day, no sign of DED with OSDI score d" 12.5 and TBUT > 10 seconds, willing to involve in this study and sign the informed consent.The exclusion criteria were subjects with chronic disease and systemic and topical medication, history of contact lens wear, subjects with ophthalmologic surgery in previous six months, subjects with eye anatomy abnormalities (eyelid disorders, cornea, sclera), eye disease other than refractive disorder, and meibomian gland dysfunction (MGD).

All samples were given questionnaire, underwent interviewed, ophthalmology examination, and tears biochemistry examination. The subjective complaints and symptoms were evaluated with OSDI questionnaire (Ocular Surface Diseases Index). Visual acuity, biomicroscope examination, Schirmer test I, TBUT (tears break-up time) were performed.Tear samples collection were using Schirmer's strip that placed at the temporal canthus of each eye. The strips were then removed from the eyes, and the amount of wetting in millimetres was recorded by observing the edge of moisture on the printed millimetre marks.

After collecting the tears, schirmer paper was put into a 0.5 ml Eppendorf tube that has been perforated and then put into a larger Eppendorf tube (1.5 ml) as described by Posa et al (2013).[17] The next procedure was centrifuged the tubes at 13,000 rpm within 5 minutes to get a liquid tear sample. IL-10 concentrations were measured quantitatively by Enzyme-LinkedImmunosorbent Assay (ELISA). This procedure was based on peptides-antibody capturedmechanism which can detect minimum level of IL-10 is 8pg /mL taken from IL-10curve concentration from Bioassay technology laboratory of human IL-10 ELISA kit.

## RESULTS

A total of 46samples have been investigated. There were 25 subjects with DED (case group) and 21 subjects without DED (control group).Table 1 shows the subject's baseline characteristics.

The proportion of male (54.3%) in this study was higher than female (45.7%). The mean age of subjects was 27.43 ± 4.47 years old. Schirmer test examined in both eyes (left and right) and presented in the table 2.Most of the samples are highly educated, from total of 45 subject we found 23 subjects (50.0%) was Diploma, 22 subjects (47.83%) was bachelor, and only 1 subject was senior high school (2.17%).

The distribution of computer use in the study subjects was homogenous with mean length of computer usage was 6.63 ± 2.16 hours. As can be seen from Table 1 that37 subjects (80.43%) claimed Computer Vision Syndrome (CVS) symptoms with the most complaint were eye or ocular complaints. It reflected from the mean of OSDI score of 16.24 with minimum score was 0 and maximum score was 56.25.Schirmer and TBUT examinations were carried out to all samples. The mean value of the Schirmer test for the study subjects was 29.78 ± 8.32 mm in the right eye and 29.50 ± 9.50 mm in the left eye. The mean of TBUT value was 12 seconds in the right eye and 10 seconds in the left eye with TBUT range 4 – 22 seconds.

**Table 1.** Baseline Characteristics of The Sample

| Characteristics | | N (%) | Mean ± Deviation standard |
|---|---|---|---|
| Sex | | | |
| Male | 25 | (54.3%) | |
| Female | 21 | (45.7%) | |
| Age (year) | | | 27.43 ± 4.47 |
| Education | | | |
| Senior high school | 1 | (2.17%) | |
| Diploma | 23 | (50.00%) | |
| Bachelor | 22 | (47.83%) | |
| OSDI score | | | 16.24 (0 – 56.25) |
| Schirmer I (mm) | | | 29.78 ± 8.32 |
| TBUT (second) | | | 12 (5 – 22) |
| CVS-DEDsymptoms | | | |
| No | 9 | (19.56%) | |
| Yes | 37 | (80.43%) | |
| Computer using (hours) | | | 6.63 ± 2.16 |

**Tabel 2.** Odds Ratio of IL-10 between case group and control group

| Dry Eye Disease | IL-10 Normal | IL-10 High | p | OR | 95% Confidence Interval Lower | 95% Confidence Interval Upper |
|---|---|---|---|---|---|---|
| Case | 3 | 22 | 0.046 | 4.308 | 0.965 | 19.236 |
| Control | 7 | 14 | | | | |

**Tabel 3.** T- Independent Test TBUT, Schirmer, and OSDI Score Between two groups

| | p | 95% Confidence Interval Lower | Upper |
|---|---|---|---|
| TBUT | | | |
| Right eye | 0.00 | 3.621 | 7.182 |
| Left eye | 0.00 | 1.686 | 6.198 |
| Schirmer I | 0.95 | 5.7 | 6.06 |
| OSDI | 0.017 | 1.56 | 15.25 |

*p< 0.05 = significantly different between case and control groups

Interleukin-10 (IL-10) levels were grouped into 2: high IL-10 and normal IL-10. The normal IL-10 cutoff point according to IL-10 curve concentration from human IL-10 ELISA kitwas classified into normal IL-10 levels (8 - 212 pg / mL) and high IL-10 level (212 pg/ mL). Bivariate analysis using a chi-square test to find the odds ratio of IL-10 level to DED found that high IL-10 level was higher in case group than the control group p <0.05 (p = 0.046). Table 2 shows that 4-hour / day computer users who have high IL-10 levels (> 212 pg / mL) have a 4.3 times greater risk of DED compared with computer users who have normal IL-10 levels (OR = 4.3; IK 95% = 0.965 - 19,236; p = 0.046).

In addition to the bivariate analysis group, an independent t-test was conducted to compare Schirmer, TBUT, and OSDI scores between the case group and the control group. The results of the independent t-test showed that the mean of Schirmer value between case and control groups did not differ significantly (p = 0.95), while the mean of TBUT score and OSDI score between case and control group were significantly different with right eye TBUT p= 0.00 (95% CI = 3,621 - 7,182), left eye TBUT p = 0.00 (95% CI = 1,686 - 6,198), and OSDI p = 0.017 (95% IK = 1.56 - 15.25).

## DISCUSSION

This study involved both healthy and DED computer users who regularly work with computer for 4 hours/ day. The diagnosis of DED was made after the examination has been carried out. In this study, our samples were at productive age and we found 37 (80,43%) samples were report CVS with DED symptoms.

The prevalence of DED based on symptom ranged between 14,4 and 24,4% in Asian population. The study in South East Asia showed the highest prevalence rates of symptomatic DED. The prevalence found ranging from 20 – 52,4%, except two study in Singapore, which only showed a prevalence of 6.5% and 12.3% among Singapore population.[18]Uchino et al., (2013) reported high prevalence of DE symptoms among VDTusers, predominantly in young adult,[19] while study in US showed at least 15-47 million people have CVS and at least 14-23% of computer users might have different degrees and types of CVS symptoms.[5]

There were two major interconnected factors that contributed to the signs and symptoms of CVS, which were near work at the computer, less than 20 in and long- hour at the computer (intense work more than 4 hours per day).[5] In this study, average of computer use was 6.63 ± 2.16 hours, which is twice than the recommendation use that can aggravate the signs and symptoms of CVS.

The ocular surface tissues contain a variety of cytokines and different subsets of regulatory T cells to reduce inflammation-induced pathology in the lacrimal functional unit. The anti-inflammatory cytokine in ocular surface includes IL-10, TGF-â, IgA, IL-RA, etc play role in ocular immune homeostasis.[ÿþ]IL-10 has function to inhibit allergic and inflammatory events in all organs including in ocular surface. In normal tissues, anti-inflammatory cytokines of IL-35 and IL-10 have a very low levels of expressions. The tear levels of IL-10 in normal human tear were between 5 and 100 pg/mL. Il-10 showed slight increases in the morning and the late evening. We take the samples at the office hours to avoid the diurnal rhythm and can characterized the process that influenced by ocular response from VDT use.[20]

In study conducted by Hos et al. (2015) shows the tear concentration and secretion of IL-1á, TNF-á, and IL-1 â were increased but Interferon-ã, Interleukin-2, Interleukin-4, and IL-10 were undetectable in the tear fluid of either strain of mice before or after experimentally induced dry eye.[19]In our study, the case group with higher level of IL-10 have 4 times risk of DED than the case group with normal IL-10 level. The increased of the IL-10 might be showed the mechanism of regulation from the stress on the ocular surface to keep homeostasis condition that still well maintained in younger people. In dry eye, IL-10 seems to play anti-inflammatory roles which secretion controlled bymacrophages. A single component change can eventually break down the homeostasis of the ocular surface microenvironmentcan promote the vicious cycle of the DED.[20]

This study limitation was that we did not measure the level of pro inflammatory cytokines to know whether there is loss of homeostasis that impaired the ocular surface and give the symptoms of DED. The small number ofsamples and cross-sectional study also contributed to the results, that

maybe have different result as if the measurements were done in several times of the day.

## CONCLUSION

Early diagnosis of DED is important, biochemical changes that can often occur before any signs of DED can be potential as tear biomarkers.The levels of these biomarkers may useful to distinguish between the risk of DE among computer users with CVS symptoms. This study concluded that high levels of IL-10 in tears increased risk ofDE 4.3 times higheramong computer users 4 hours/day. Therefore, inflammatory cytokines such as IL-10 has important roles in the immunopathogenesis of the DED. The individual assessment of this biomarker and its comparation with anti-inflammatory cytokines could be useful in the diagnosis and management of DE especially in high risk group. Further study regarding the role of other cytokines in DED with prospective design and larger samples is needed.

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