



Effect of hormonal contraceptives on the ocular surface and the tear film

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ABSTRACT

Submitted: 2020-05-21
Accepted : 2022-06-28

Dry eye syndrome (DES) is a common multifactorial disease of the tears and ocular surface associated with sex hormones. Hormonal contraception is a risk factor for DES, but its relationship with DES exacerbations in women of childbearing age is still unclear. This study aimed to evaluate changes of the tear film and ocular surface of young women using the hormonal contraceptive agent. It was a case-control study involving 56 healthy women aged 20 to 45 y.o. Subjects was divided into two groups i.e. the hormonal contraceptives group and the control group without hormonal contraceptive. Subjects were interviewed with the ocular surface disease index (OSDI) questionnaire. Tear secretion and tear stability were measured using Schirmer's I test and fluorescein tear break-up time test (TBUT). Ocular surface impression cytology with cellulose acetate filter paper was taken from inferonasal bulbar conjunctiva and was stained with periodic acid-Schiff (PAS) and counterstained with hematoxylin and eosin (HE). No significantly decrease in tear secretion and tear stability in the hormonal contraceptives group compared with the control groups was observed ($p > 0.05$). However, a statistically significant decrease in goblet cell density and conjunctival epithelium metaplasia was observed, where 25% of the hormonal contraceptives group had an abnormal impression cytology result compared with the control group ($p < 0.05$). The hormonal contraceptives group also had a higher OSDI score than the control group, although it was not statistically significant ($p > 0.05$). The hormonal contraceptives group had a significant effect on the ocular surface in which it induced squamous metaplasia and inflammation of conjunctival cells and the reduced number of goblet cells ($p < 0.05$). The slightly decreased tear film volume and stability accompanied by an increase in OSDI score found in the hormonal contraceptives group support the possibility of hormonal contraceptive use as one of the risk factors in the occurrence of dry eye syndrome.

ABSTRAK

Sindrom mata kering (*dry eye syndrome/DES*) adalah penyakit multifaktorial umum dari air mata dan permukaan mata yang terkait dengan hormon seks. Kontrasepsi hormonal merupakan faktor risiko DES, tetapi hubungannya dengan kejadian eksaserbasi DES pada wanita usia subur belum jelas. Penelitian ini bertujuan mengkaji perubahan yang terjadi pada lapisan air mata dan permukaan mata pada remaja putri yang menggunakan kontrasepsi hormonal. Penelitian menggunakan kasus-kontrol yang melibatkan 56 wanita berusia 20 sampai 45 tahun. Subjek dibagi menjadi dua kelompok, kelompok kontrasepsi hormonal dan kelompok kontrol tanpa kontrasepsi hormonal. Semua partisipan telah diwawancarai dengan kuesioner *ocular surface disease index* (OSDI). Sekresi air mata dan stabilitas air mata diukur menggunakan tes Schirmer I dan fluorescein *tear break-up time* (TBUT). Sitologi impresi permukaan okular dengan kertas saring selulosa asetat diambil dari konjungtiva bulbar inferonasal dan diwarnai dengan periodic acid-Schiff (PAS) dan hematoxylin dan eosin (HE). Terjadi penurunan sekresi air mata dan stabilitas air mata tidak signifikan pada kelompok kontrasepsi hormonal dibandingkan dengan kelompok kontrol ($p > 0,05$). Namun demikian, terdapat

Keywords:

hormonal contraceptives;
tear secretion and stability;
sex hormones;
impression cytology;
dry eye

penurunan densitas sel goblet dan metaplasia epitel konjungtiva yang signifikan, yakni 25% kelompok kontrasepsi hormonal memiliki hasil sitologi impresi abnormal dibandingkan dengan kelompok kontrol ($p < 0,05$). Kelompok kontrasepsi hormonal juga memiliki skor OSDI yang lebih tinggi dibandingkan kelompok kontrol, meskipun tidak signifikan ($p > 0,05$). Kontrasepsi hormonal memiliki efek yang signifikan pada permukaan okular yang menginduksi metaplasia skuamosa dan inflamasi sel konjungtiva dan penurunan jumlah sel goblet ($p < 0,05$). Volume dan stabilitas lapisan air mata yang sedikit menurun disertai dengan peningkatan skor OSDI yang ditemukan pada pengguna kontrasepsi hormonal mendukung kemungkinan penggunaan kontrasepsi hormonal sebagai salah satu faktor risiko terjadinya sindrom mata kering.

INTRODUCTION

Dry eye syndrome (DES) is a common multifactorial disease of the tears and the ocular surface that can significantly diminish visual function and quality of life. Dry eye prevalence increases in both genders with age. However, the higher incidence among females after menopause suggested a role of sex hormones in its onset.^{1,2} Sex hormones imbalance may be related to the onset and development of ocular surface disease and DES. The hormonal imbalance that influences tear physiology may be manifested with the use of hormonal contraceptives or menopause when significant changes from the normal hormonal state occur.³⁻⁵

Hormonal contraceptives are a known risk factor for DES. However, there is still an unclear association between hormonal contraceptive use and exacerbation of DES in women of childbearing age. Hormonal contraceptives are supposed to alter the androgen, estrogen, and progesterone serum level and exert a significant influence on the lacrimal gland, meibomian gland, and ocular surfaces. They may play an essential role in the pathogenesis of DES.^{5,6} He *et al.*,⁷ reported that women who use hormonal contraceptives regularly have two times risk to develop DES than women who irregularly use. However, other studies reported contradictory outcomes about hormonal contraceptive effects on the tear film. Several studies reported that hormonal contraceptives does not

significantly affect tear physiology.^{6,8-10} Another studies reported that women using oral contraceptives were more likely to experience DES than women not using it.^{3,11,12} The effect of hormonal contraceptives on the ocular surface was still not apparent. Versura *et al.*⁴ reported that the impairment of tear production and stability, ocular surface dryness, and inflammation are significantly related to hormonal fluctuations in the menstrual cycle, especially in patients with DES. It was also reported a significant decrease of goblet cell density in the follicular phase in women with and without dry eye but more pronounced in dry eye patients. Furthermore, it was suggested that the influence of estrogen in this finding since estrogen due to the dominant hormone in the follicular phase. These findings suggested that the hormonal imbalance due to other factors, such as hormonal contraceptives that could also affect the structural and functional changes in the cornea and conjunctiva cells. These structural and functional changes can be detected by impression cytology, which is a reliable and valuable tool for detecting structural and functional changes in ocular surface cells with a non-invasive and easy-to-perform procedure.^{13,14} The inconclusive results from the previous study about hormonal contraceptives effects on the tear film need further study. The studies concerning the effect of hormonal contraceptives on the ocular surface, especially in goblet cells density and conjunctival cells metaplasia are limited. This study aimed to analyse the changes

of tear film regarding its secretion and stability, and the ocular surface, in terms of the goblet cells density and conjunctival cells metaplasia, in young women using hormonal contraceptive agents. This study will give new insight and information about the safety of hormonal contraceptive use on the ocular surface.

MATERIALS AND METHODS

Subjects

It was a case-control study done involving the outpatient clinic of the Andalas University Hospital, Padang, West Sumatra, Indonesia. The protocol of study was approved by the Research Ethic Committee, Faculty of Medicine, Andalas University with the reference number of ethical clearance: 594/KEP/FK/2019. Informed consent was obtained from each respondent before the study started. A total of 56 healthy women of childbearing ages, from age 20 until 45 y.o. were involved in this study. The sample size was calculated using the unpaired numerical, analytical study design formula. From the formula, the sample size for this study was 28 people for each group, i.e., the hormonal contraceptives group and the control group without the hormonal contraceptive.

Sample for the hormonal contraceptives group were women who regularly used hormonal contraceptives for at least one year, including combined oral contraceptives, injections, or subdermal implants. The participants were taken from the primary health facilities in the vicinity of the university hospital. Samples for the control group were women who had never used any hormonal contraceptive methods, and the participants were taken from the employee of the university hospital. The age inclusion criteria for both groups were from 20 until 45 y.o. Detailed history taken included personal data,

the use of hormonal contraceptives and other forms of contraceptives, the type of hormonal contraceptive used, duration of use, visual complaint history, general health history, and systemic medication uses. A detailed ocular examination was done in the outpatient clinic to rule out any ocular surface and anterior segment abnormality.

Sample excluded in this study were women with ocular surface infection and inflammation, women with eyelid abnormalities (entropion, ectropion, and lagophthalmos), women with diabetes and autoimmune diseases, and current consuming oral β -blocker, antihistamines, and psychoactive drugs. Women with a history of ocular trauma or surgery, contact lenses wearers, pregnant women, women under topical eye drops, menopausal and post-menopausal women were also excluded.

Data collected include the severity of dry eye disease, measured by the ocular surface disease index (OSDI) questionnaire (OSDI; Allergan, Inc, Irvine, CA); the production of the tear film, measured by Schirmer's I test; the stability of tear film, measured by tear break-up time test; and goblet cell density and conjunctival cell morphology, measured by impression cytology procedure.

Data collection

Demographic information, history of using hormonal contraception, contact lens wear, ocular history, past medical and surgical history, current systemic and ophthalmic medications were obtained by patient self-report. In the first step, all participants were interviewed with the OSDI questionnaire (α : 0.92 (CI 95%: 0.89-0.94)).¹⁵ The scores obtained were then calculated to determine dry eye disease severity as normal, mild, moderate, and severe. Then, all participants underwent a slit-lamp examination to rule out

eyelid and ocular surface abnormalities.

After slit-lamp examination, Schirmer's I test was performed to measure total tear secretion. Schirmer's strip (Whatman filter paper 41) was gently put at the junction of the middle and outer 2/3 of the lower lid, taking care not to touch the cornea or eyelashes. Participants were asked to close their eyes during the procedure. The strip was then removed 5 min after insertion. The wet portion of the strip measured in mm started from the bent strip.

The tear break-up time test (TBUT) was conducted after the Schirmer's I test was completed. The test used a fluorescein dye strip that gently swept over the conjunctival surface, and the participants were instructed to blink several times. Then, the participants were instructed to refrain from blinking, and fluorescein stained-tear film was observed on a slit lamp with cobalt blue light to notice the appearance of the dark spot or streak. The time elapsing between the eye-opening after the last blinking and the appearance of the first dark spot or streak was measured with a stopwatch. Three successive measurements were taken, and the mean value was calculated.

The final step was the conjunctival cells specimen collection for impression cytology, taken according to procedures described by Singh.¹⁴ One drop of local anesthetic was instilled into the eye, and excessive tear fluid and medication were wiped away. Cellulose acetate filter paper was trimmed into a 5 mm strip with one square end and one tapering end. An eye speculum was inserted, and participants were asked to look up. The filter paper was applied to the conjunctiva on the inferonasal area. The filter paper was smoothed onto the ocular surface by applying gentle pressure with a glass rod. The paper was allowed to remain in contact with the ocular surface for approximately 5–10 sec and then peeled off with forceps.

The filter paper was then fixed in a solution containing glacial acetic acid, formaldehyde, and ethyl alcohol in a 1:1:20 volume ratio for approximately 10 min. The specimen was then sent to the pathology laboratory for routine histological staining of impression cytology specimens with periodic acid Schiff with hematoxylin counterstain. The readings were then graded according to Scheffer and Tseng's classification.¹⁶ Grade 2 or more is considered abnormal.

Statistical analysis

Differences in measurements between the two groups were assessed with t-tests for OSDI scores, Schirmer's I test, and TBUT results. Differences in impression cytology grades and dry eye grades based on the OSDI score were assessed with Fisher's exact test for categorical factors. A p value < 0.05 was considered as significant.

RESULTS

This study involved 56 females in the reproductive age group, divided into two groups; each group consisted of 28 females. The demographic profile is shown in TABLE 1, which demonstrates that the mean age for both groups is similar. However, the mean age of the control group is younger than the hormonal contraceptives group. Ethnicity in both groups was similar, with Minangkabau as the dominant ethnicity. Respondents in the control group had a higher level of education than the hormonal contraceptives group. Most respondents in the hormonal contraceptives group work as housewives, while most respondents in the control group work as employees. Only one person in the hormonal contraceptives group had a smoking history for 15 years, and she smokes one cigarette a day.

TABLE 1. Characteristics of respondents in both groups.

Characteristics	Group	
	Hormonal contraceptives	Control
Mean age (mean \pm SD y.o.)	35.96 \pm 6.563	31.57 \pm 5.607
Ethnicity [n (%)]		
• Minangkabau	26 (92.85)	27 (96.43)
• Java	1 (3.575)	1 (3.57)
• Sunda	1 (3.575)	-
Education [n (%)]		
• Elementary school	1 (3.58)	-
• Junior high school	4 (14.28)	-
• Senior high school	23 (82.14)	3 (10.71)
• Diploma	-	15 (53.57)
• Bachelor	-	9 (32.14)
• Postgraduate	-	1 (3.58)
Employment [n (%)]		
• Employee	1 (3.57)	22 (78.57)
• Paramedic staff	-	6 (21.43)
• Housewife	21 (75.00)	-
• Microbusiness	6 (21.43)	-
Smoking history [n (%)]		
• Yes	1 (3.57)	
• No	27 (96.43)	28 (100)

TABLE 2 shows the type of hormonal contraceptives used. Injection hormonal contraceptive was the commonest hormonal contraceptive used. The injection used is three monthly hormonal contraceptives containing depot medroxyprogesterone acetate (DMPA), a progesterone hormone. Combined oral contraceptives were the second most commonly used hormonal contraceptive agent containing ethinylestradiol and levonorgestrel, a combination of estrogen and progestins. The subdermal

implant was the less common method chosen for hormonal contraceptives, which contained levonorgestrel. Combined oral contraceptives had the most prolonged duration of use among other types of hormonal contraceptives.

TABLE 3 shows that 25% of the hormonal contraceptives group participants had an abnormal impression cytology result compared with none in the control group who had an abnormal result, which is statistically significant.

TABLE 2. The type of hormonal contraceptives used

Hormonal contraceptive agent	Frequency [n (%)]	Duration of use (mean ± SD years)
Combined oral contraceptives	7 (25)	9.14 ± 5.336
Injection	16 (57.1)	5.69 ± 4.922
Subdermal implant	5 (17.9)	2.40 ± 1.517

TABLE 3. Impression cytology grades between groups.

Impression cytology grade	Group		p
	Hormonal contraceptives [n (%)]	Control [n (%)]	
Grade 0	6 (21.4)	16 (57.1)	0.008
Grade 1	15 (53.6)	12 (42.9)	
Grade 2	4 (14.3)	0 (0.0)	
Grade 3	3 (10.7)	0 (0.0)	

TABLE 4 shows a slight decrease in the mean Schirmer value on the hormonal contraceptives group compared with the control group but not statistically significant. There is also a slight decrease in mean TBUT value in the hormonal contraceptives group compared with the control group but

not statistically significant. TABLE 4 also shows that participants who used injection hormonal contraceptives had the lowest mean value of Schirmer test and TBUT compared to combined oral contraceptives and subdermal implant hormonal contraceptive users, but this was not statistically significant.

TABLE 4. Mean value of the Schirmer test and TBUT between groups.

Tear film parameters	Hormonal contraceptives	Control	p	Combined oral contraceptives	Injection	Subdermal implant	p
Schirmer	22.71 ± 13.34	24.18 ± 10.06	0.645	31.29 ± 6.99	19.19 ± 13.27	22.00 ± 17.00	0.133
	10.76 ± 4.98	12.35 ± 5.79		12.68 ± 4.93	8.99 ± 4.46	13.73 ± 5.09	

TABLE 5 shows that the hormonal contraceptives group had a higher OSDI score than the control group, but this was not statistically significant. It also shows that more participants with

moderate and severe dry eyes in the hormonal contraceptives group than in the control group, but this was not statistically significant.

TABLE 5. OSDI scores and DES severity, based on the OSDI score.

Variable	Group		p
	Hormonal contraceptives	Control	
OSDI score	24.34±23.672	17.25±17.66	0.210
DES severity [n (%)]			
• Normal	12 (42.9)	14 (50.0)	0.229
• Mild	7 (25.0)	11 (39.3)	
• Moderate	8 (28.6)	3 (10.7)	
• Severe	1 (3.6)	0 (0.0)	

DISCUSSION

Tear physiology is affected by hormones from the hypothalamic-pituitary-gonadal axis, mainly due to the influence of sex steroids (androgens, estrogens, and progestins) and pituitary hormones. Hormonal influences on tear physiology may be manifested with the use of hormonal contraceptives or during menopause when significant changes from the normal hormonal state occur.^{6,17} All type of hormonal contraceptives inhibit proliferation of ovarian follicles, resulting in anovulation and a decrease in circulating estrogen. Decreased serum oestradiol could have adverse effects on estrogen-sensitive tissues, including the conjunctiva. The conjunctival proliferation is disturbed and more susceptible to the development of squamous metaplasia and inflammation and exhibits a reduced number of goblet cells.^{2,4,17,18}

The impression cytology results in this study (TABLE 3), graded according to Scheffer and Tseng's classification,¹⁶ showed that women who used hormonal contraceptives had more abnormal results, which were 14.3% on Grade 2 (marked loss of goblet cells without keratinization (FIGURE. 1. A)) and 10.7%

on Grade 3 (Marked loss of goblet cells with early and mild keratinization (FIGURE. 1. B)). Furthermore, this result differs significantly from the women in the control group, who all had normal results (Grade 0 (FIGURE. 1. C) and Grade 1 (FIGURE. 1.D)). Murube and Rivas¹⁹ found a significantly decreased goblet cell density with an increase in dry eye severity. Shrestha *et al.*,²⁰ found similar results that 49.2% of dry eye cases showed decreased or absent goblet cell density compared with only 26.3% of normal eyes showing decreased or absent goblet cells ($p < 0.001$). This study found a higher prevalence of moderate and severe dry eye in the hormonal contraceptives group (32.2%) compared with the control group (10.7%) (TABLE 5). This finding also corresponded with the higher prevalence of abnormal grade of impression cytology results and decreased goblet cells in 25% of participants in the hormonal contraceptives group (TABLE 3). The loss of goblet cells and mild keratinization of conjunctiva found in these participants may correlate with the slight decrease of tear film parameters in the hormonal contraceptives group compared with the control group.

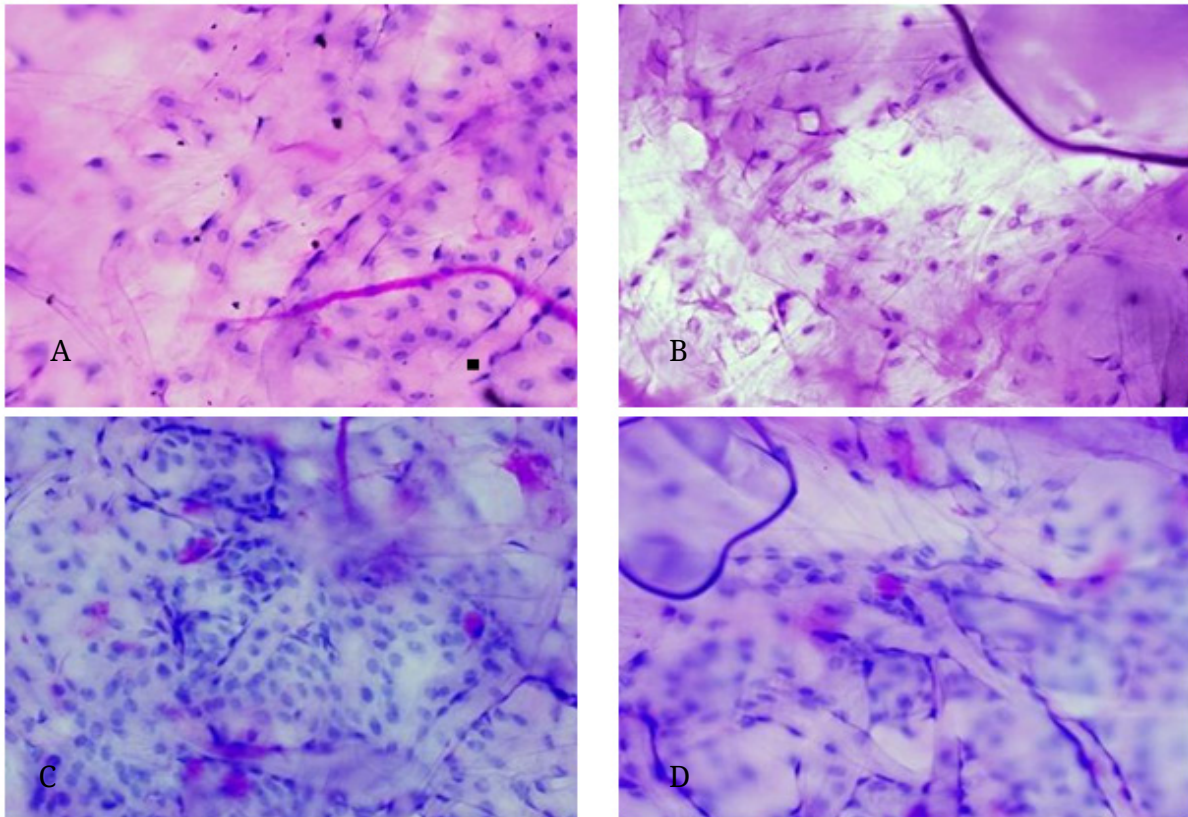


FIGURE 1. Conjunctiva impression cytology photomicrograph (magnification 40x, Scale bar, 10 μ m). A. Grade 2 of impression cytology in the hormonal contraceptives group shows marked loss of goblet cells without keratinization. B. Grade 3 of impression cytology in the hormonal contraceptives group shows considerable loss of goblet cells with early and mild keratinization. C. Grade 0 of impression cytology in the control group shows normal conjunctival epithelium with a scattered moderate number of goblet cells. D. Grade 1 of impression cytology in the control group shows early loss of goblet cells without keratinization.

The results of this study (TABLE 4) also show a decrease in tear film production and stability in women who use hormonal contraceptives, based on the Schirmer test and TBUT, respectively, although statistically not significant ($p=0.645$ and $p=0.274$) compared with women who not use it. Another study by Tomlinson *et al.*,⁶ who carried out a study on the effects of oral hormonal contraceptives on tear physiology, reported no significant difference in tear secretion and osmolarity between women on oral hormonal contraceptives and the control group. A similar result was also reported by Idu *et al.*,¹⁰ which found no significant differences in tear

secretion and tear stability between women on injectable hormonal contraceptives and women not using hormonal contraceptives. Sharma *et al.*,³ reported different results that they found a significant decrease in tear secretion in women using oral hormonal contraceptives (13 ± 3 mm) than in the control group (31 ± 7 mm/5min) with $p=0.00$. Nevertheless, they do not find any significant difference in TBUT value between the two groups. Women in the hormonal contraceptives group, who used injection hormonal contraceptives had the lowest tears secretion and tears stability than women who used combined oral

contraceptives or subdermal implant hormonal contraceptives, but this was not statistically significant (TABLE 4). Women using combined oral contraceptives had better tear film parameters than women using other hormonal contraceptives, although theirs had the most prolonged duration of use. Other studies found that oral contraceptives did not affect Schirmer I test, tear break-up time (TBUT) test, and tear osmolarity value.^{8,9,21} So, despite a decrease in serum androgen level, this does not correlate linearly with tear film parameters. We assumed that the serum level decrease was not too significant, so it did not cause an alteration in androgen bioactivity, which causes the androgen hormone to be unaffected in the lacrimal and meibomian glands.^{22,23} This normal androgen bioactivity is seen in normal values of tears secretion and stability in women who used combined oral contraceptives, three monthly injections, and subdermal implant hormonal contraceptives. A study by Sharma *et al.*,³ also found that the serum androgen level (testosterone and DHEA) was slightly decreased by 12% in the hormonal contraceptives group compared with the control, but the tear film parameters are still within normal limits. However, this study does not measure the androgen serum level of all participants, so the relationship between the type of hormonal contraceptives and androgen bioactivity on the lacrimal and meibomian gland is not yet confirmed. The mean OSDI score of both hormonal contraceptives and the control group belongs to the mild grade of dry eye severity. However, the mean OSDI score and dry eye severity are higher in the hormonal contraceptives group (TABLE 5), even not statistically significant. The OSDI is a validated questionnaire that only measures the frequency of dry eye symptoms, but its correlation with clinical signs and tear film parameters is still weak.²⁴⁻²⁷ Garhöfer *et al.*,²⁸ and

Song *et al.*,²⁹ found weak negative correlation between OSDI score and tear film parameters such as Schirmer and TBUT tests. The higher OSDI score in the hormonal contraceptives group corresponds to its Schirmer and TBUT tests value reduction. However, Chen *et al.*,⁹ found that neither oral contraceptives use nor estrogen dose of oral contraceptives appeared to have a meaningful effect on tear osmolarity and dry eye symptoms measured by OSDI. Concerning the significant result of the abnormal grade of impression cytology in women using hormonal contraceptives in this study, accompanied by decreased tear film parameters and increased OSDI score, hormonal contraceptives could negatively impact tear film physiology and promote the occurrence of DES.

A small sample size is considered a limitation in this study, as a larger sample size could give more significant results. The lack of measurement of androgen and estrogen serum levels is another limitation because we can not do linear regression analysis between tear film parameters and androgen and estrogen serum levels to find its correlation. Further study with a large sample size, long-term follow-up, and hormonal serum level measurement should be done to establish hormonal contraceptives' impact on the tear film and ocular surface epithelium cells.

CONCLUSION

Hormonal contraceptives had a significant effect on the ocular surface in which they induced squamous metaplasia and inflammation of conjunctival cells and reduced the number of goblet cells. The slightly decreased tear film volume and stability accompanied by increased OSDI score found in hormonal contraceptives users support the possibility of contraceptive use as one of the risk factors in the occurrence of DES.

ACKNOWLEDGEMENT

This study was supported in part through a grant from the Faculty of Medicine Andalas University, Padang, West Sumatra.

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