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# Tear function tests and conjunctival impression cytology before and after hormone replacement therapy in postmenopausal women

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PURPOSE. *To investigate the effect of hormone replacement therapy (HRT) on postmenopausal tear function and the conjunctival epithelium.*
METHODS. *Schirmer I-Jones test, tear film break-up time (BUT), and impression cytology findings were analyzed in 34 eyes of 17 women who were at least two years postmenopausal and not taking HRT. This series of tests was repeated after three months on HRT.*
RESULTS. *The patients' average age was 53.82 ± 3.6 years, and the mean time postmenopause was 35.29 ± 11.59 months. There was no significant difference in the Schirmer I-Jones test results before and after three months of HRT ($p > 0.05$). However, the BUT ($p < 0.05$) and impression cytology ($p < 0.05$) findings were significantly affected by HRT.*
CONCLUSIONS. *HRT may alleviate postmenopausal dry eye symptoms by increasing goblet cell density. (Eur J Ophthalmol 2003; 13: 337-42)*

KEY WORDS. *Menopause, Tear function tests, Impression cytology, Hormone replacement therapy*

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## INTRODUCTION

Although many factors are involved in the etiology of dry eye syndrome, the higher incidence in women over 50 years of age suggests that physiological changes associated with menopause play a role. After menopause, circulating estradiol (E2) levels are approximately 10-20 pg/mL, and most of this E2 derives from the peripheral transformation of estrone (E1). The source of estrone is androstenedione and, since postmenopausal blood androstenedione levels are roughly half the premenopausal levels, E2 after menopause is also far below premenopausal levels (1). Dermal thickness and the amount of collagen in the skin both decrease with age, but these changes can be prevented with estrogen replacement therapy (2). This treatment reduces the degradation of collagen while increasing its quality (3). One large series showed that the use of estrogen lessens dry skin and wrinkles (4). Changes in the maturity index of the conjunctival epithelium parallel to hormonal changes during the menstrual cycle indicate that sex hormones affect this tissue layer (5). The postmenopausal increase in keratoconjunctivitis sicca (KCS) and the lower incidence of this condition in women who are taking systemic estrogen replacement therapy suggest that lack of estrogen may play a role (6, 7).

The purpose of this study was to investigate the effects of hormone replacement therapy (HRT) in postmenopausal women by testing tear function and examining the conjunctival epithelium.

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## MATERIALS AND METHODS

The study included 34 eyes of 17 women examined in the Obstetrics and Gynecology Department of Başkent University Faculty of Medicine. All had menopausal complaints and had experienced natural menopause at least two years earlier. The exclusion criteria were as follows:

1. Systemic therapy that might affect endocrine and ocular findings
2. Previous HRT
3. Surgically induced menopause
4. Any ocular disease
5. Previous ocular surgery

The patients all underwent a routine eye examination before and after starting HRT and tear function tests were done in the following order.

1. *Tear film break-up time (BUT):* No topical anesthesia was administered. A drop of 2% sodium fluorescein solution was instilled with an eyedropper and, using the cobalt blue light of the slit-lamp, the time between the last blink and the first appearance of a dark spot was measured. This procedure was repeated three times, and the average (in seconds) was recorded.

2. *Schirmer I-Jones test:* Using an eyedropper, one drop of 0.5% proparacaine hydrochloride was instilled in each eye for topical anesthesia. In a dimly lit room, a standard Schirmer strip was placed inside the margin of the inferolateral third of the lower lid, taking care not to let the paper come into contact with the cornea. After 5 minutes, the strip wetting was measured (in millimeters).

3. *Impression cytology:* After topical anesthesia to the ocular surface, samples were taken from the inferior and temporal conjunctiva of each eye studied. Nitrocellulose (Sartorius AG.37070, Göttingen, Germany) filter paper with a pore diameter of 0.45 µm was used to collect the specimens. Filter papers were cut into 3 x 4 mm rectangles that were applied with gentle pressure for 2 to 3 seconds. Once the paper had completely adhered to the conjunctiva, the edge was lifted and it was removed. Samples were kept in 95% ethanol at +4 °C and stained with hematoxylin and eosin-periodic acid-Schiff (PAS). They were examined under the light microscope by the same pathologist without knowledge of the clinical status. Each sample was graded from 0 to 3 according to Nelson et al (8), as outlined below.

*Grade 0:* The epithelial cells are small and round with eosinophilic-staining cytoplasm. The nuclei are large and basophilic, with a nucleus-to-cytoplasm ratio of 1:2. The goblet cells are abundant, plump, and oval and have an intensely PAS-positive cytoplasm.

*Grade 1:* The epithelial cells are slightly larger and more polygonal and have eosinophilic-staining cytoplasm. The nuclei are smaller, with a nucleus-to-cytoplasm ratio of 1:3. There are fewer goblet cells but they maintain their plump, oval shape with an intensely PAS-positive cytoplasm.

*Grade 2:* The epithelial cells are larger and polygonal, occasionally multinucleated, with variably staining cytoplasm. The nuclei are small, with a nucleus-to-cytoplasm ratio of 1:4 to 1:5. The goblet cells are markedly decreased in number and are smaller and less intensely PAS positive, with poorly defined edges.

**TABLE I -** RESULTS (MEAN ± STANDARD DEVIATION [SD]) OF THE SCHIRMER I-JONES TEST, TEAR FILM BREAK-UP TIME (BUT), AND IMPRESSION CYTOLOGY BEFORE AND AFTER HORMONE REPLACEMENT THERAPY (HRT)

| Test | | Before HRT | After HRT | p value |
|---|---|---|---|---|
| Schirmer I-Jones test (mm/5 min) | | 10.35 ± 5.7 | 11.73 ± 5.5 | 0.094 |
| BUT (sec) | | 8.05 ± 2.1 | 9.76 ± 2.0 | 0.001 |
| Cytology grading | Temporal conjunctiva | 1.11 ± 0.7 | 0.85 ± 0.7 | 0.037 |
| | Inferior conjunctiva | 1.05 ± 0.8 | 0.70 ± 0.6 | 0.012 |

Grade 3: The epithelial cells are large and polygonal with basophilic-staining cytoplasm. The nuclei are small, pyknotic and, in many cells, absent. The nucleus-to-cytoplasm ratio is greater than 1:6. Goblet cells are absent.

Patients were prescribed daily oral doses of 0.625 mg conjugated estrogen + 2.5 mg medroxyprogesterone acetate (Premelle 2.5 mg, Wyeth, Turkey) for three months. After this period, the procedures described above were repeated.

### *Statistical analysis*

A paired-samples t-test was used to compare the findings before and after HRT. A p value less than 0.05 was considered statistically significant.

## RESULTS

The patients' average age was 53.82 ± 3.6 years (range 50-60) and the mean time postmenopause was 35.29 ± 11.59 months (range 24-48 months). The mean findings for the Schirmer I-Jones, BUT, and cytological investigations before and after three months of HRT are shown in Table I. The Schirmer I-Jones test scores were slightly higher after HRT, but this difference was not significant. However, at the end of HRT, BUT was significantly longer than at baseline BUT.

Table II summarizes the cytological grading before and after three months of HRT, listed according to the specimen grade and location. Before HRT, the mean grades (reflecting goblet cell count) of the specimens taken from the temporal and inferior conjunctiva were 1.11 ± 0.7 and 1.05 ± 0.8, respectively; after HRT, they were 0.85 ± 0.7 and 0.70 ± 0.6. Both the temporal and the inferior quadrant conjunctival impression cytology grades after three months of HRT were significantly different from the pre-HRT findings ($p = 0.03$ and $p = 0.01$, respectively) (Figs. 1 and 2). Conjunctival metaplasia decreased and goblet cell count increased while the patients were on HRT, most notably in the inferior quadrant. The numbers of goblet cells increased significantly during HRT in 22 eyes (64.7%): in the temporal quadrant in 10 (29.4%) and in the inferior quadrant in 12 eyes (35.2%) (Figs. 3, a and b).

## DISCUSSION

The close relationship between sex hormones and the eye is demonstrated by the ocular discomfort and blurry vision experienced by some women who use oral contraceptives and similar problems in pregnant women who wear contact lenses (9). Gans et al (10) detected no estrogen or progesterone receptors in their immunohistochemical study of the ocular surface. However, ten years later, Wickham et al (11), using the reverse transcriptase-polymerase chain reaction, found androgen, estrogen, and progesterone receptor mRNA in a number of different tissues, including lacrimal glands, meibomian glands, and the palpebral and bulbar conjunctiva.

Kramer et al (5) were the first to show that the conjunctiva is an estrogen-sensitive structure. They found a correlation between premenopausal estrogen levels and the maturation index of the conjunctival epithelium, and reported that during ovulation, when estrogen levels are highest, the conjunctival epithelium is also at its most advanced state of maturity. They found no such difference in the maturation of the conjunctival epithelium in postmenopausal women. Further, improvement

**TABLE II -** NUMBERS (%) OF CYTOLOGIC SPECIMENS OF EACH GRADE IN EACH LOCATION (temporal and inferior conjunctiva) BEFORE AND AFTER HORMONE REPLACEMENT THERAPY (HRT)

| Grade | Before HRT | | After HRT | |
|---|---|---|---|---|
| | Temporal conjunctiva | Inferior conjunctiva | Temporal conjunctiva | Inferior conjunctiva |
| 0 | 7 (21%) | 9 (26%) | 11 (32%) | 14 (41%) |
| 1 | 16 (47%) | 15 (44%) | 17 (50%) | 16 (47%) |
| 2 | 11 (32%) | 9 (26%) | 6 (18%) | 4 (12%) |
| 3 | 0 | 1 (3%) | 0 | 0 |

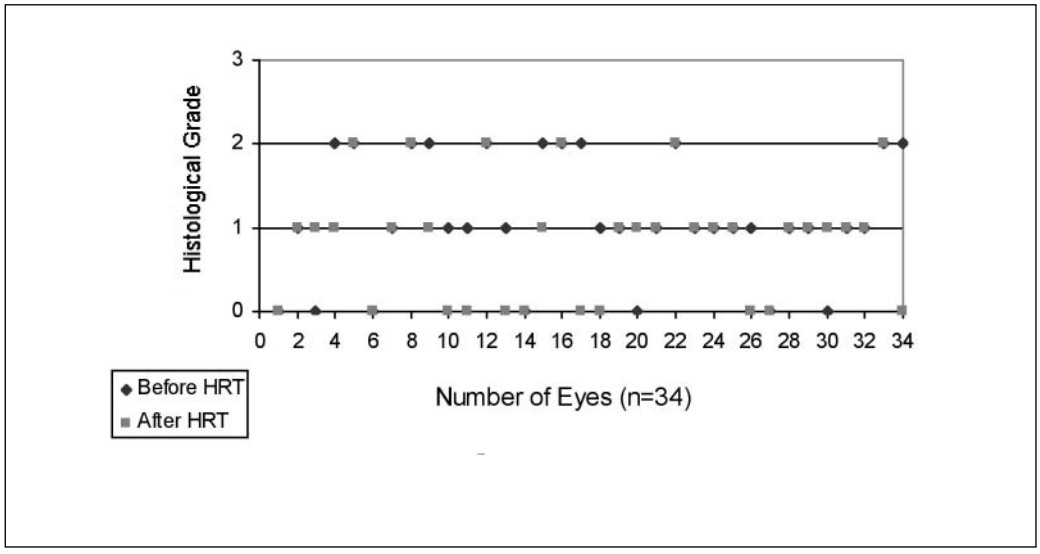


**Fig. 1 -** *Response of the temporal conjunctiva to hormone replacement therapy (HRT).*

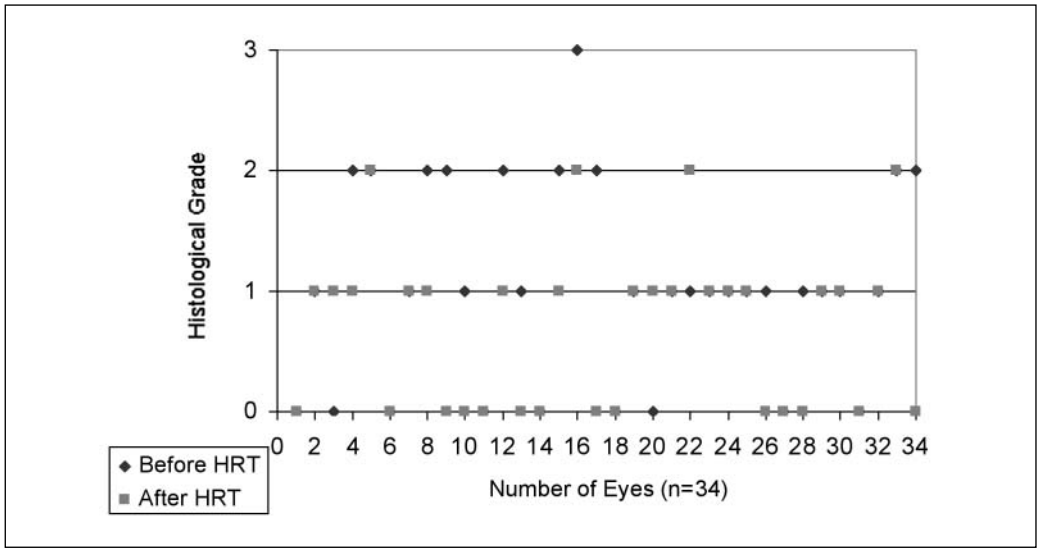


**Fig. 2 -** *Response of the inferior conjunctiva to hormone replacement therapy (HRT).*

of KCS with estrogen therapy indicates that estrogen affects the dryness of the eye (12). In the study carried out by Schaumberg et al (13), the use of progesterone/progestin in combination with estrogen resulted in lower risks of dry eye syndrome than with estrogen alone in postmenopausal women. They did not exclude contact lens use or other medications that might have led to an increased frequency of dry eye syndrome.

Tear film homeostasis involves delicate hormonal and neuronal regulatory mechanisms. The eye appears to be a target organ for sex hormones, particularly the androgens, as they modulate the immune system and trophic functions of the lacrimal glands, and the functioning of the meibomian glands (14). There are reports of changes in tear function with age, including decreases in the quantity and stability of tears (15). In an animal model, sex hormone deficiency was reported to lead to decreased tear quantity and impaired tear function (16).

In our caselist, Schirmer I-Jones values were slightly increased after three months of HRT, but the change was not significant. One investigation comparing subjects taking antiandrogen therapy for prostatic indications with age-related controls showed that the treated patients had significantly higher frequencies of tear

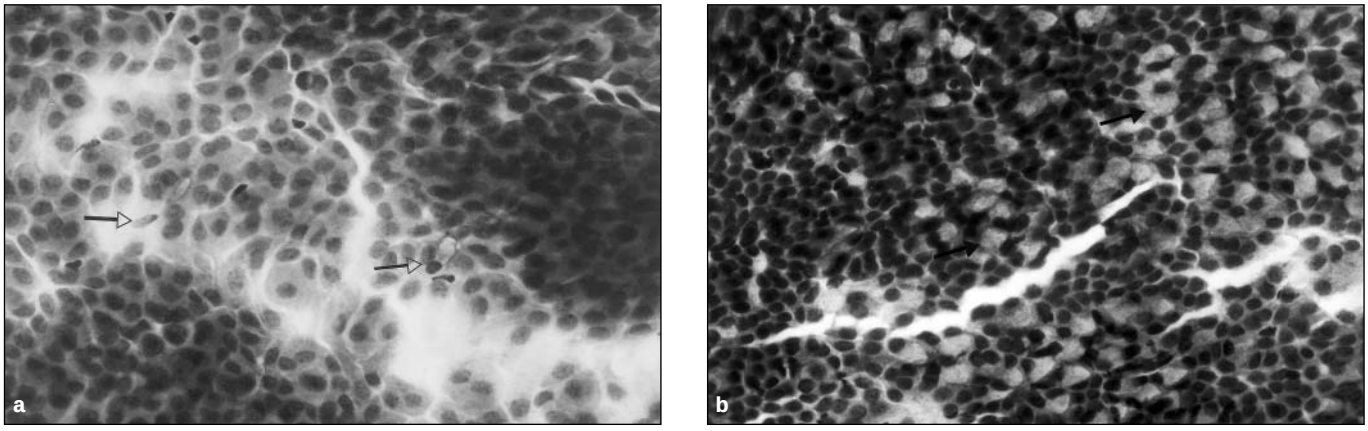


**Fig. 3 -** *Inferior quadrant conjunctival cytological specimens before and after hormone replacement therapy (HRT) in the same patient.* **a)** *Impression cytology before HRT, grade 2. The epithelial cells are larger and polygonal, occasionally multinucleated, with variably staining cytoplasm. The nuclei are small, with a nucleus-to-cytoplasm ratio of 1:4 to 1:5. The goblet cells (arrows) are markedly decreased in number and are smaller (periodic acid-Schiff-hematoxylin, original magnification x100).* **b)** *Impression cytology after HRT, grade 0.The small, round epithelial cells have large nuclei with a nucleus-to-cytoplasm ratio of 1:2. Goblet cells (arrows) are abundant, plump, and oval, with intensely periodic acid-Schiff (PAS)-positive cytoplasm (PAS-hematoxylin, original magnification x100).*

film debris, abnormal tear film meniscus, irregular posterior lid margins, conjunctival tarsal injection, and meibomian gland dysfunction (17). Sullivan et al (18) suggested that treatment of the ocular surface with androgens promotes lacrimal and meibomian gland function, and alleviates both aqueous-deficient and evaporative dry eye.

Objective findings on goblet cell density can be obtained through histologic examination of conjunctival epithelial cells collected by applying a Millipore filter (19). Abdel-Khalek et al (20) found a decrease in goblet cell numbers in a population over 80 years of age. However, another study that used conjunctival impression cytology found no correlation between age and goblet cell count (21). Goblet cells are most numerous in the tarsal conjunctiva and the inferonasal bulbar conjunctiva (22). We found that the number of goblet cells increased more in the inferior than in the temporal conjunctiva after HRT.

Artificial tear preparations provide only temporary relief of dry eye. These complaints increase in the postmenopausal period, suggesting that the dry eye symptoms may be related to conjunctival metaplasia and goblet cell loss. A number of studies have reported that BUT decreases and tear film stability deteriorates with decreasing goblet cell density (8, 23). Vavilis et al (24) identified differences in the cytological maturation of the conjunctival epithelium in patients who had used transdermal estradiol for four months after menopause.

Our findings showed that the cytological phases after three months on HRT were consistent with the BUT changes. The effect of estrogen in preventing goblet cell loss was consistent with that reported in the literature. The significant differences in BUT and cytology suggest that HRT may be a therapeutic option in the treatment of postmenopausal dry eye syndrome.

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