

# The Study of Tear Function, Central Corneal Thickness and Corneal Endothelial Cell Count in Postmenopausal Hormone Replacement Therapy

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**ABSTRACT : Objective :** To study various ocular parameters in the postmenopausal women who were treated and not treated with hormone replacement therapy (HRT).

**Study design :** Observational study

**Settings :** Ramathibodi Hospital, Bangkok, Thailand

**Patients :** This study was conducted on 82 healthy postmenopausal women, aged 48 to 69 years (mean age  $55.0 \pm 4.8$  years). All women were divided into two groups, 38 women were treated with hormone replacement therapy and 44 women were not treated with hormones.

**Measures :** Demographic characteristics, tear secretion, stability of the tear-film, central corneal thickness and corneal endothelial cell count.

**Results :** We found that the average corneal endothelial cell count in HRT group ( $2822.0 \pm 299.7$  cells/mm<sup>2</sup>) was more than the non-HRT group ( $2690.8 \pm 252.8$  cells/mm<sup>2</sup>) (P-value = 0.003). A slightly more thickness was observed in the HRT group as the average central corneal thickness in the HRT group was  $566.7 \pm 38.3$   $\mu$ m and in the non-HRT group was  $556.1 \pm 35.1$   $\mu$ m but the difference did not reach the statistical significance (P-value = 0.067). Tear secretion and stability of tear-film were not different between the two groups (P-value = 0.334 and P-value = 0.959).

**Conclusion :** The HRT might have a positive effect on the corneal endothelial cell count but no effect on central corneal thickness, tear secretion and tear stability in postmenopausal women. **Thai J Ophthalmol 2004 ; July-December 18(2) : 121-127.**

Keratoconjunctivitis sicca (dry eye syndrome) was estimated to affect up to 40% of the elderly female population.<sup>1,2</sup> It had long been established that dry eye is particularly common in women and frequently manifested following the onset of menopause.<sup>3,4</sup> Among 19 ocular complaints listed by Metka et al<sup>5</sup> in postmeno-

pausal women, 7 were related to tear function, and the second most commonly reported symptom was "feeling of dryness". Thus, impairment in the regulation of lacrimal secretion seemed to play a prominent role in the pathogenesis of postmenopausal ocular symptomatology. Recent researches<sup>6-9</sup> had demonstrated that sex steroids (an-

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drogen, estrogen and progesterone) exerted a significant influence on the structure and function of numerous ocular tissues. Many studies on hormone replacement therapy also demonstrated the positive effect on ocular functions eg. tear production, tear stability, IOP and corneal thickness in postmenopausal women. However, some reports<sup>10,11</sup> showed that HRT did not appear to alter the incidence of dry eye symptoms. The results of these studies had still not been settled conclusively.

The aim of this study was to assess the ocular parameters in terms of tear secretion, tear stability and central corneal thickness in the postmenopausal woman who were treated and not treated with HRT for the more basic data to support the benefit of HRT. The study was also to study the effect of HRT on corneal endothelial cell count which had never been reported before.

### Materials & Methods

Postmenopausal women involved in this study were participating in protocols approved by the Ramathibodi Hospital Ethics Committee and all signed written informed consent forms.

The eighty-two consecutive postmenopausal women recruited from the postmenopausal clinic, department of OB-GYN, Ramathibodi Hospital from September 2002 to May 2003, aged 48 to 69 years (mean age  $55.0 \pm 4.8$  years). Thirty-eight women were treated with oral HRT and forty-four women were not treated with hormones.

The eligibility criteria for this study included the women who had natural postmenopausal status and those

in the treated group could receive hormones continuously for at least 3 months before the study. The exclusion criteria were the ocular diseases that would effect the main outcome measurement such as meibomian gland dysfunction, chronic inflammation of conjunctiva, superficial pemphigoid, neurotrophic keratitis, blepharitis and irregular corneal surface. The patients who used to wear contact lenses, received any eye surgery or excimer laser, had diabetic retinopathy, toxic thyroid goiter, Sjögren's syndrome and had any medication that would result in tear production eg. antidepressants were also excluded.

Demographic information were recorded, including age, age of menarche, age of menopause and history of medication intake. For the treated group, the time they started hormone therapy and duration were recorded. Each woman underwent an examination of tear secretion, tear stability, central corneal thickness and endothelial cell count measurement.

Schirmer I test is the tear secretion measurement. It measures both basal and reflex tear secretion. A Whatman No. 41 filter paper size 5 x 35 mm was placed between the middle and lateral one-third of the lower fornix. The test was done under ambient light. The patient was asked to look straightforward and to blink normally during the course of the test. We measured the wetting value of the paper over a period of 5 minutes.

#### Tear breakup time (TBUT)

This test measures the stability of the tear film by using normal saline solution dropped over the fluorescein strip and applied in the lower fornix gently. After sweeping the overflow tear, asking the patient to blink three times and hold the eyelids open after the

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last blinks. The tear film was observed under cobalt-blue filtered light of the slitlamp microscope and the time that elapsed between the last blink and appearance of the first break in the tear film was recorded with a stopwatch (a break was seen as a dark spot over the corneal surface). In this study we performed the test 5 times consecutively for the average value. TBUT was abnormal if the value was less than 10 seconds.

### Central corneal thickness

The central corneal thickness was measured by using corneal topography device (ORBSCAN). The patient was asked to look straightforward and hold the eyelids open for a few seconds in front of this device.

### Corneal endothelial cell count

We dropped the topical anesthesia and artificial tear into the patient's eyes 30 seconds before measurement with specular microscope (EM-1000, Tomey corporation.) We applied an applanating tip to contact the center of cornea surface gently until we obtained a good specular image.

### Statistical analysis

Statistical analysis was performed by Chi square

test for evaluation of tear secretion and tear stability and by student's t-test for central corneal thickness and endothelial cell count between the two groups. We used the statistical software program stata version 7.0. Statistical significance was set at P-value < 0.05.

## Results

Between September 2002 and May 2003, 82 patients were enrolled in the study, 38 received HRT and 44 did not. They all had both eyes examined. Table 1 represents the demographic characteristics of the women of the two groups. We found that age, age of menarche, age of menopause were very similar with no statistically significant difference (P-value > 0.05).

Table 2 shows the values of the tear secretion, tear stability, central corneal thickness and endothelial cell count of the two groups. Schirmer I test indicated a deficiency in total tear secretion. Most individuals with normal secretory rates will saturate at least 10 mm or more of the paper strip. If the secretory rates were less than 5 mm, this would represent the dry eye condition. In the HRT group, the Schirmer I test < 5 mm, 5-10 mm

**Table 1** Relationship of demographic characteristics among postmenopausal women with and without use of hormone replacement therapy (HRT)

Characteristic	HRT N = 38 subjects	Non HRT N = 44 subjects	P-value
Age (years) mean ± SD	55.2 ± 5.4	55.1 ± 4.5	0.924
Menarche (years) mean ± SD	13.9 ± 1.4	13.8 ± 1.7	0.703
Menopause (years) mean ± SD	48.7 ± 3.0	49.7 ± 2.8	0.132

**Table 2** Results of Schirmer I test, tear break up time, central corneal thickness and corneal endothelial cell count between HRT and non-HRT groups

	HRT (n = 76 eyes)	Non HRT (n = 88 eyes)	P-value
Schirmer I test			
< 5 mm	8 (10.5%)	4 (4.6%)	0.334
5-10 mm	8 (10.5%)	9 (10.2%)	
> 10 mm	60 (79.0%)	75 (85.2%)	
TBUT			
< 10 sec	55 (72.4%)	64 (72.7%)	0.959
≥ 10 sec	21 (27.6%)	24 (27.3%)	
Central corneal thickness (µm)	566.7 ± 38.3	556.1 ± 35.1	0.067
Corneal endothelial cell count (cells/mm <sup>2</sup> )	2822.0 ± 299.7	2690.8 ± 252.8	0.003

and > 10 mm were 10.5%, 10.5% and 79.0%, respectively while in the non-HRT group were 4.6%, 10.2% and 85.2%, respectively. No statistically significant difference was found between the two groups (P-value = 0.334).

The tear stability was measured with the fluorescein break up time. If the value were less than 10 seconds, it would be the dry eye condition. In the HRT group, 55 of 76 eyes (72.4%) had the TBUT values less than 10 seconds compared to the non-HRT group that 64 of 88 eyes (72.7%) had TBUT values less than 10 seconds. There was no statistically significant difference between the two groups (P-value = 0.959).

The average central corneal thickness of the HRT

groups was 566.7 ± 38.3 µm while the non-HRT group was 556.1 ± 35.1 µm. There was a slightly more thickness in the HRT group but no statistically significant difference (P-value = 0.067).

The average endothelial cell count in the HRT group was 2822.0 ± 299.7 cells/mm<sup>2</sup> while the non-HRT group was 2690.8 ± 252.8 cells/mm<sup>2</sup>. We found that there was statistically significant difference between the two groups (P-value = 0.003).

### Discussion

There was no beneficial effect of HRT on tear secretion and tear stability in the postmenopausal women in our study. Our results supported Mathers et al<sup>11</sup>

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who evaluated 128 women for symptoms of dry eye, HRT status and tear function (osmolarity, tear flow, tear volume, Schirmer test) and they concluded that HRT did not appear to alter the incidence of dry eye symptoms or physiologic dry eye in older women prior to or during menopause. Moreover, a recent study<sup>12</sup> in which 25,665 postmenopausal women provided information on the use of HRT compared with those who did not receive it. The data suggested that women who used HRT, particularly estrogen alone, were at increased risk of dry eye syndrome. They discussed on the basic research suggested that sex hormone levels might influence both the lacrimal and meibomian gland. Laboratory and preliminary clinical studies suggested that whereas androgen had a beneficial influence on lacrimal and meibomian gland function, estrogen might play a role in exacerbating dry eye syndrome. Given their findings as well as the known inhibitory effects of estrogen on ocular sebaceous glands, further study of the effects of estrogen on the function of the meibomian gland (a large sebaceous gland containing estrogen receptors) will be interesting and need to be ascertained. Whether the effects of HRT will have beneficial effect on ocular function or not, the gynecologist still prescribes HRT to the postmenopausal women for other reasons not for dry eye syndrome while the ophthalmologist still orders artificial tear to decrease the dry eye symptoms. HRT will be prescribed more easily in the postmenopausal women with dry eye if there are more researches, which support the benefit of HRT.

There was an increase in central corneal thickness after 3 and 6 months of HRT but did not reach a statis-

tical significance in the study by Affinito et al.<sup>10</sup> Indeed, several studies performed on women in their fertile period<sup>13-15</sup> and in pregnancy<sup>16</sup> showed that central corneal thickness might be positively related to estrogen and progesterone serum levels. In our study, the average central corneal thickness in HRT group was slightly thicker than the non HRT group but did not reach a statistical significance as in Affinito's study. We did not measure the central corneal thickness in the same patient before and after HRT. Therefore, we plan to follow the central corneal thickness in all postmenopausal women again 1 year later to see whether there will be any change or not.

Finally, the average corneal endothelial cell count in the HRT group was clinically significant more than the non-HRT group. There was no any study of corneal endothelium cell count in the postmenopausal hormone replacement therapy and no evidence of any relationship between corneal endothelial cell and HRT before. In this study, we did the corneal endothelial cell count because we would like to complete the corneal examination and we did not expect any difference between the two groups. However, HRT might have a positive effect on the corneal endothelial cell count as our results but it could not be fully described because it was still controversial with regards to estrogen, androgen and progesterone receptor in the cornea. Further studies are needed to conclude the relationship between corneal endothelial cells and sex hormones. Because we did not measure the corneal endothelial cell count in the same patient before and after HRT, we have to follow the corneal endothelial cell count 1 year later to see any change in both groups.

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# การศึกษาผลของฮอร์โมนทดแทนที่มีต่อการทำงานของน้ำตา ความหนาของกระจกตาและจำนวนเซลล์ในชั้น Endothelium ของกระจกตาในหญิงวัยหมดประจำเดือน

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**บทคัดย่อ**      **วัตถุประสงค์** : เพื่อศึกษาการทำงานของน้ำตา ความหนาของกระจกตา และจำนวนเซลล์ในชั้น endothelium ของกระจกตาในหญิงวัยหมดประจำเดือนที่ได้รับและไม่ได้รับฮอร์โมนทดแทน

**วิธีการศึกษา** : เป็นการศึกษาแบบ observational study ในผู้หญิงวัยหมดประจำเดือน 82 ราย อายุระหว่าง 48-69 ปี เฉลี่ย  $55.0 \pm 4.8$  ปี โดยแบ่งออกเป็นสองกลุ่มคือ กลุ่มที่ได้รับฮอร์โมนทดแทนจำนวน 38 ราย และกลุ่มที่ไม่ได้รับฮอร์โมนทดแทน จำนวน 42 ราย หญิงวัยหมดประจำเดือนทุกคน จะได้รับการซักประวัติเกี่ยวกับอายุที่เริ่มมีประจำเดือน อายุที่หมดประจำเดือน วัดระดับน้ำตา วัดความคงสภาพของน้ำตา วัดความหนาของกระจกตา และนับจำนวนเซลล์ในชั้น endothelium ของกระจกตา

**ผลการศึกษา** : จำนวนเซลล์ในชั้น endothelium ของกระจกตาในกลุ่มที่ได้รับฮอร์โมนทดแทนมีค่าเฉลี่ย  $2822.0 \pm 299.7$  เซลล์ต่อตารางมิลลิเมตร ซึ่งมากกว่าในกลุ่มที่ไม่ได้รับฮอร์โมนทดแทนที่มีค่าเฉลี่ย  $2690.8 \pm 252.8$  เซลล์ต่อตารางมิลลิเมตร  $P\text{-value} = 0.003$  และพบว่าความหนาของกระจกตาในกลุ่มที่ได้รับฮอร์โมนทดแทนมีค่าเฉลี่ย  $566.7 \pm 38.3$  ไมครอน ซึ่งหนากว่าในกลุ่มที่ไม่ได้ฮอร์โมนทดแทนที่มีค่าเฉลี่ย  $556.1 \pm 35.1$  ไมครอน อย่างไม่มีนัยสำคัญทางสถิติ  $P\text{-value} = 0.067$  สำหรับระดับน้ำตา ความคงสภาพของน้ำตา ไม่มีความแตกต่างกันระหว่างสองกลุ่ม  $P\text{-value} = 0.334$  และ  $P\text{-value} = 0.959$

**สรุป** : ฮอร์โมนทดแทนอาจมีผลในทางบวกต่อจำนวนเซลล์ในชั้น endothelium ของกระจกตา แต่ไม่มีผลต่อความหนาของกระจกตา ระดับน้ำตา และความคงสภาพของน้ำตาในหญิงวัยหมดประจำเดือน **จักษุเวชสาร 2547 ; กรกฎาคม-ธันวาคม 18(2) : 121-127.**

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