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**Examining the Relationship between Hormone Therapy  
(HT) and Dry-Eye Syndrome in Post-Menopausal Women:  
A Cross-Sectional Comparison Study**

Dissertation in partial fulfillment of the requirement for the award of  
**Doctorate Degree in Medicine** conferred by Faculty of the University  
of Saarland, Germany

Zur Erlangung des Grades eines Doktors der Medizin  
Der Medizinischen Fakultät  
Der Universität des Saarlandes

2016

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**Geboren am: 25.03.1981 in Abu Dhabi United Arabic Emirate**

To my wife and my new born child Mohamed

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# List of Symbols, Abbreviations and Nomenclature

ANOVA	ANOVA- Analysis of variance
BMI	BMI-Body mass index
CRA	CRA-Central retinal artery
DES	DES-Dry eye syndrome
DHEAS	DHEAS- Dehydroepiandrosterone sulfate
IDEEL	IDEEL- Impact of dry eye on everyday living
IOP	IOP-Intraocular pressure
HT	HT-Hormone therapy
OSDI	OSDI-Ocular surface disease index
PCA	PCA- Posterior ciliary artery
POLA	POLA- Pathologies Oculaires Liees a l Age
SANDE	SANDE- Symptom Assessment in Dry Eye questionnaire
SHBG	SHBG- Sex-hormone binding globulin
TFBUT	TFBUT- Tear film breakup time
UAE	UAE-United Arab Emirates
SKMC	SKMC-Sheikh khalifa medical city

# 1. Summary

**Objective:** Postmenopausal women undergo severe physical, psychological, and physiological changes that require treatment if they are to perform daily functions. While hormone therapy (HT) has gained popularity as a ministrations method for menopause-related complaints, its potential to increase DES risk requires further investigation. DES can impair normal functioning of individuals. As such, it is appropriate to research the link between DES and sex hormones, as well as between HT and DES—mainly in women that have reached menopause. Findings from the current research will bring new insights into the literature in obstetrics and gynecology regarding the relationship between DES and HT.

To examine the relationship between hormone therapies (HT) and DES in women will provide patients and medical professionals with a balanced review of HT from the ocular perspective. By producing and presenting factual results, this study will educate women and healthcare professionals regarding the credibility of HT. The study will also open avenues for further studies either to develop solutions regarding the risks of HT or to develop more effective treatments related to menopause.

**Methods:** 177 postmenopausal women currently under HT (estrogen alone and estrogen and progesterone) and 183 women not under HT participated in a validated IDEEL questionnaire-based survey study that was conducted in 30 gynecological clinics.

**Outcome Measures:** 1) severity of symptom levels (based on the OSDI index levels of normal, mild, moderate and severe) identify the extent of the association between DES and changes in sex hormones; and 2) comparison of severity of symptom levels between women under HT and not under HT establish the relationship between HT and DES in postmenopausal women.

**Results:** Based on severity levels obtained through the ODSI formula, only 8.9% of the respondents belonged to normal range of the OSDI index. Analysis of variance (ANOVA) indicates the following: i) significant variation in the severity levels of DES across women not under HT, under HT (estrogen alone) and HT (estrogen and progesterone) [ $F(2, 357)= 974.186$ ,  $p=.000$ ]; ii) significant variation in the severity levels of DES based on dosage levels (<1 mg/day and >1 mg/day) for women under HT (estrogen alone) and HT (estrogen and progesterone) [ $F(2, 357)= 302.513$ ,  $p=.000$ ]; and iii) significant variation in the severity levels of DES based on duration levels 12, 24 and 36 months) for women under HT (estrogen alone) and HT (estrogen and progesterone) [ $F(3, 356)= 218.266$ ,  $p=.0001$ ].

**Conclusion:** The current study findings suggest that HT is not a protective factor against the severity of DES in postmenopausal women.



## 2. Zusammenfassung

Nach der Menopause durchlaufen Frauen schwere körperliche und Veränderungen, die manchmal eine Therapie benötigen. Die Hormontherapie (HT) hat an Popularität gewonnen, um die postmenopausale Beschwerden zu reduzieren. Gleichzeitig ist es wichtig, die Risiken solcher Therapien zu untersuchen. Der Zusammenhang zwischen DES im Rahmen der postmenopausalen Beschwerden sollte in Bezug auf die Wirkung von HT und Sexualhormonen untersucht werden.

Zu diesem Zweck wurden postmenopausale Patientinnen und medizinisches Fachpersonal in Hinsicht auf Augenbeschwerden untersucht und gebeten, eine Bewertung der Hormontherapie abzugeben.

Methoden: 177 postmenopausalen Frauen derzeit unter HT (Östrogen allein und Östrogen und Progesteron) und 183 Frauen, die nicht unter HT waren, nahmen an einer validierten Fragebogenerhebung teil, die in 30 Frauenkliniken durchgeführt wurde.

Zielparameter: 1) Schwere Symptom (auf der Grundlage der OSDI Indexniveaus normal, leicht, mittel und schwer) klassifizieren das Ausmaß der Assoziation zwischen DES und Veränderungen der Geschlechtshormone; und 2) Vergleich der Schweregrade der Symptome zwischen Frauen unter HT und ohne HT verdeutlichen die Beziehung zwischen HT und DES bei postmenopausalen Frauen.

Ergebnisse: Basierend auf die durch die ODSI Formel gewonnenen Schweregrade hatten nur 8,9% der Befragten einen normalen OSDI Index. Die Varianzanalyse (ANOVA) gibt die folgenden Ergebnisse: i) bedeutende Änderung der Schweregrade von DES gegenüber Frauen aus der unbehandelten Kontrollgruppe gegenüber der Gruppe unter HT (Östrogen allein) und HT (Östrogen und Progesteron) [ $F(2, 357) = 974,186, S. < 0,0001$ ]; ii) bedeutende Änderung der Schweregrade von DES in Abhängigkeit von der Dosierung ( $< 1 \text{ mg / Tag}$  und  $> 1 \text{ mg / Tag}$ ) für Frauen unter HT (Östrogen allein) und HT (Östrogen und Progesteron) [ $F(2, 357) = 302,513, p < 0,0001$ ]; und iii) wesentliche Veränderung der Schweregrade von DES unter HT (Östrogen allein) und HT (Östrogen und Progesteron) in Bezug auf die Therapiedauer 12, 24 und 36 Monate [ $F(3, 356) = 218,266, p < 0,0001$ ].

Fazit: Die aktuellen Ergebnisse der Studie legen nahe, dass HT nicht als Schutzfaktor gegen das DES bei postmenopausalen Frauen wirkt.

## **3. Introduction**

### **3.1 Dry-Eye Syndrome**

Dry-eye syndrome during menopause is defined as a group of defects on the ocular surface that produce abnormalities in the tear film. These aberrations to the tear film result in perpetual visual disturbances and discomfort with eventual damage of the ocular surface (Friedman 2010).

The dry-eye classification criteria present two main categories of the disease: evaporative-tear deficiency that results from the disturbance of the tear-film and leads to instability; and aqueous-tear deficiency that results from a reduced volume of tears produced by the lacrimal glands. The dry-eye condition is marked with increased evaporation and drying of the ocular surface and a higher rate than the produced tears can replenish. In both cases, hormonal changes have been cited as the precursors of dry eye. Hormonal changes affect the ocular tissues and the composition of tears the eyes produce. During menopause, the risk of dry-eye syndrome appears to increase with increased estrogen production. Findings by Brignole et al. (2008) suggest that increased risk of dry-eye disease results from increased exposure to estrogen during hormone therapy.

#### **3.1.1 The Ocular Surface**

The interface between the eye and the environment constitutes the ocular surface. The ocular surface is made up of the conjunctival epithelia and the cornea, the neural arc that connects the glands and epithelia with trigeminal nerve centers, the tear film that ensures the eye is lubricated and moist, in addition to lubricating accessory and main lachrymal glands. Together, these components work to give the eye a high-quality optical view while protecting the ocular structures. Eye components and their functions have been elaborated by Tseng and Tsubota (2011).

#### **3.1.2 Pre-ocular tear film and ocular surface epithelia**

A thin layer of fluid protects the conjunctival and corneal epithelia that have a number of biological functions. These functions provide have a primary defense purpose against noxious and foreign agents (Barney, 2002). The tear film has two components: an aqueous surface with dissolved particles of mucin, and superficial layers that limit evaporation (Altintas et al., 2004). When estrogen- and progesterone-rich medication contacts this surface, there is an effect on the internal mucins that may result in increased or reduced surface tension of the liquid, making the

epithelial table wet in normal persons or dry in persons with dry-eye syndrome. Under normal sex-hormone balance, the ocular surface and the pre-ocular tear film maintain the eye's surface integrity. Imbalance of the sex hormones, however, may compromise the stability of the ocular system (Watanabe et al., 2005).

### **3.1.3. Epithelial cell surface functions**

The limbo-stromal micro-environment regulates the differentiation of the epithelial cells that TAC and stem cells find on their differentiation pathways. In most of the cornea pathologies, the sub-epithelial basal membrane constitutes a preventive mechanism against the inflammatory cytokines as the stromal fibroblasts control the composition of the stroma (Fini, 2009). The ocular surface functions as a unit to protect the eye both from the mechanical effects of the environment and from hormonal changes that lead to eye conditions during menopause (Altintas et al., 2004).

### **3.1.4. Maintenance of tear film stability**

The composition of tear-film is important for ensuring the stability and proper visual acuity of the eye. The sex hormone often interferes with the meibomian gland, which secretes superficial tear lipids. The sex hormones also interfere with accessory glands that discharge protein-containing and electrolyte-containing tears by reducing their secretions, and hence may cause dry-eye syndrome. Therefore, the glands play a central role in the system in ensuring equal distribution of tears across the ocular surface. Equal distribution is realized through eye blinking. Blinking also triggers the meibomian gland to produce tears through the nasolacrimal drainage system (Doane, 2001).

### **3.1.5. Protective mechanism of Neuro-anatomic integration**

Externa adnexa, the lids, and surface epithelia are harmonized through neuronal nervous reactions (Tseng and Tsubota, 2011). In most cases, the neural arc integrates the function of the other components and uses the ophthalmic nerve. It controls the major lachrymal gland in producing the aqueous fluid, thereby manipulating the defensive mechanism by influencing tear production (Barney, 2002). In the second neural reflex, the motor-neuron branch from the study controls, and the facial nerve regulates lid closure and blinking. In this way, it influences the

protective layer, where tears spread on the ocular surface and its outflow. Synthesizing this process is the last incorporation of the two reflexes as demonstrated by the tear turnover or clearance. During sex-hormone imbalance, the process is impaired and the tears evaporate at a higher rate, leaving a dry eye and a poorly drained ocular surface. The condition may worsen if there is prolonged use of HT in postmenopausal women, especially with the increased use of estrogen-based hormone therapy (Barney, 2002).

### **3.1.6 Chronic Inflammation and Dry-Eye**

Any sex-hormone imbalance can contribute to an unstable or scarce pre-ocular tear film. In addition, sex-hormone imbalance—e. g., high levels of estrogen—can contribute to a large volume of unrefreshed tears that soluble mediators generate (Avis et al., 2010). Due to its prolonged condition in women in their postmenopausal years, scholars have reached a consensus that DES is a chronic condition that involves both cellular mediators and soluble mediators (Brignole et al., 2000; Brignole et al., 2008; Baudouin, 2001). This conclusion is based on a number of documented research findings. For example, dry eye is marked by increased intracellular-adhesion molecule-1, epithelial cells, expression of immune-activation molecules, and tears. All of these increased secretions infiltrate the inflammatory cells and appear to be highly expressed in persons with dry-eye syndrome (Brignole et al., 2000; Stern et al., 2002).

These activities are often regulated by the pro-inflammatory cytokines, for example IL-6, IL-1, and TNF- $\alpha$ . They also seem elevated in tears of persons with DES (Dinareello, 2000; Solomon et al., 2001). Commonly, they are involved in the stimulation of the inflammation cells. Moreover, TNF- $\alpha$  and IL-1 trigger conjunctival inflammatory cells to penetrate the ocular surface. This results in the production of matrix metallo-proteins enzymes (Li et al., 2011; Smith et al., 2001). The enzymes can degrade the corneal-epithelium basal membranes. The activated inflammatory aspects in the tears act in a latent form like substance P, pro-tumor necrosis factor-alpha, and pro-interleukin-1b (Sternlicht and Werb, 2011). An increased rate of apoptosis of the ocular lining epithelial cells can be significant in the initiation of dry-eye inflammation. The use of human study models (Brignole et al., 2008) and animal models (Gao et al. 2008), has contributed to the identification of a new process that can activate kinases in regulating apoptosis and inflammation (Rosette and Karin, 2006).

### **3.1.7 The Ocular Surface and Sex Hormones**

Two types of tear production have been documented: reflex and basal. Scholars consider the main lachrymal gland solely responsible for the release of the reflex tears that originate from the trigeminal pathway to induce the ocular surface stimulation. The same process may also be achieved from the stimulation of the retina using psychic emotions or high-energy light radiation (Mathers et al., 1998). The conjunctival goblet cells and accessory lachrymal gland were initially thought to be responsible for continuous and slow production of basal tears (Smith et al., 2001; Pérez-López et al., 2009). More recently, convincing evidence has come to light concerning the presence of the reflex secretion and basal secretion. As a result, tears are today considered to be secreted after neural stimulation of the glands influenced by peripheral or central systems through orthosympathetic or parasympathetic pathways (Jordan and Baum, 2008).

In the ocular surface, the primary lachrymal gland is the main immune secretory tissue. In the gland, there is often an increased number of mononuclear cells that come from different parts of the body. Their activity is largely regulated by the actions of the neuroendocrine control (Hodges and Dartt, 2003), though additional research is needed to explain the matter (Chan et al., 2006). Additional studies are also necessary to examine the role that sex hormones play on the ocular surface functioning, further complicating the matter. For instance, current knowledge is inadequate to clarify if ocular surface dysfunction is closely linked to excess production of estrogen or its inefficient production. It is also necessary to determine whether the correlation results from a deficiency in androgen or from an imbalance between androgen and estrogen.

The findings from a large, epidemiological study conducted by Schaumberg et al. (2002) confirm that women are four times more likely to develop dry-eye syndrome than men of the same age. The study considered populations above 45 years of age in the period of female life when endocrine changes impact hormone balance, resulting in the impairment of several body functions. During post-menopause, the main endocrine change results in reduced levels of estrogen secretion as the ovarian follicles degenerate. This is not because of deprivation, because some estrogen continues to be released, mainly in obese women (Carr, 1998). The decline is attributed to reduced levels of plasma androgen by (Labrie F, 1997). Additional research showed that women with premature ovarian functions show significant ocular surface damage (Smith et

al., 2004). Dry-eye syndrome can also be reported in expectant women when their level of estrogen is sustained at considerable levels (Schechter et al., 2002).

## **3.2 Dry-Eye Syndrome Therapy**

### **3.2.1 General Therapies for dry-eye syndrome**

In daily gynecologic practice, dry-eye-syndrome therapy is among the most problematic of clinical interventions. The condition is even worse in women who use hormone therapy. Today, the mechanism by which HT affects the production of normal tears is currently under scientific research. Only few molecules have been patented, although the outcomes among patients include various side effects (Baudouin, 2001). What is significant is to distinguish specific part of the tear glands that has been affected. To successfully care for patients with dry-eye conditions, importantly, individuals should stabilize each of the non-functioning components (Chan et al., 2006). However, managing the situation merely by treating the affected patients with tear substitutes may become problematic and less effective in resolving the problem. Self-prescription of drugs—specifically of astringent drops or vasoconstrictor—is thought to be a poor practice and is strictly forbidden. It is mainly self-administered to prevent eye redness. This approach can worsen the symptoms, because the intervention works to reduce the production of tears.

Commercially, several products are in the global market used to manage DES in postmenopausal women (Brignole et al., 2008). The list is being extended and enlarged by the day. Most of the medications are based on common approaches of action that has been categorized into: nutritifying the ocular surface tissue, modifying the altered tear component, stabilizing the tear structure through the reduction of surface tension, restoring the normal tear volume, and diluting the hyperosmolar tears (Pelit et al., 2003).

Commonly, synthetic tears can clean the harmful agents concentrated on the ocular surface, draining them from the ocular surface. This process contributes incidentally to low inflammation process. Anti-inflammatory medications were proposed initially to manage the dry-eye condition. Some of the hypothesized approaches use tetracyclines, corticosteroids, and cyclosporine A and administer the autologous serum (Jacobs et al., 2013). Even so, it is advised that caution be exercised when administering these medications. Patients should also be followed closely and monitored mainly for potential steroid side effects in patients. The effects of, use of, and rationale

behind topical or systematic HT for postmenopausal women with dry-eye syndrome are considered in the subsequent section.

### **3.2.2 Hormone imbalance and Dry-eye syndrome**

Androgen deficiency has been linked to dysfunctions in the Meibomian gland according to the Beaver Dam eye research. Evaporative dry eye, and tear-film instability that commonly indicate the Sjögren's syndrome that develops almost entirely in women (Klein et al., 1997). The androgen hormones have been reported to control the immune system, in addition to controlling the secretory and morphology roles of played by the lacrimal gland. Experiments on animals, such as rabbits and rats, have identified receptors for several ocular tissues for prolactin, progesterone, estrogens, and androgens. Both Lacrimal gland and meibomian gland contains mRNA androgen receptor, several enzymes, and proteins that are involved in the conversion of metabolized androgens in different androgenic forms (Hiller et al., 1997).

Some postmenopausal women with dry-eye syndrome express immunological disorders in the conjunctiva and lacrimal gland. Solberg et al. (1998) report that postmenopausal women with poor reflex tear is more likely to complain of lymphocytes and autoantibodies infiltration. This can be explained by the ocular distress that results from desiccation, resulting in a number of injured cells and release of the pro-inflammatory cytokine. Once the inflammatory trigger is triggered, autoimmune reactions take place, resulting in the exacerbation of dry-eye symptoms. Given that the hormones work to suppress the proteolytic enzymes and inflammatory factors, any hormone imbalance that results from HT therapy is potentially able to cause these proteases to become active and to worsen the dry-eye syndrome.

Even so, the specific influence of HT in postmenopausal women is not clear. This lack of clarity results in two conflicting schools of thought. One faction argues that HT increases the volume and the quality of the tear films, thus significant in moderating the impact of DES. Other scholars report that the use of HT over a long period of time worsens DES and increases the risk of disease progression. It is conceivable that HT can alleviate DES in postmenopausal women by increasing the density of goblet cells (Sperduto et al., 1990). This may be so despite the fact that the same HT worsens the symptoms of dry eye in some cases, and that in other cases the use of estrogen alone is linked to severe symptoms compared to a combination of progesterone and estrogen therapy. Results from women's health research indicate that 69% of the reported dry-eye

syndrome risk is related to post-menopause estrogen therapy, while 29% of the higher risk of the dry-eye syndrome is attributed to women taking progesterone and estrogen therapy (Kahn et al., 1997).

### **3.2.3 Hormone therapy and Tear function changes**

A recent study performed a tear sample collection and initial ophthalmic evaluation by giving subjects a daily dose of conjugated estrogens (0.625 mg) and either an incessant cyclic or combined 5 mg medroxyprogesterone acetate. The eye exam took into account a number of factors, including break-up-time evaluation, tonometry, fundus examination, Schirmer test, slit-lamp examination, and visual acuity. Findings from the study reveal that lysozyme levels and tear immune globulin A (IgA) from the gel electrophoresis were high. In addition, corneal desquamation, laxity, and conjunctival vascular congestion did not change before the experiment and after the trial. There was substantial improvement in the inflammation of the meibomian gland. No significant difference was reported in tearing, foreign-body sensation, and burning. Furthermore, no significant difference was noted in the tear functions and on Schirmer's test values. Although immunoglobulin A and lysozyme enzymes were elevated after the therapy, a significant increase was found only in the IgA levels. This implies that HT contributes to the decline in meibomian gland inflammation and to increase the levels of IgA and tear lysozyme in postmenopausal women.

### **3.2.4 Hormone therapy and Lens Opacities**

As noted in previous sections, it is still a matter of debate how the estrogen used in hormone therapies protects against lens opacities in postmenopausal women. However, studies from animal models based on age-related cataracts indicate that estrogen reduces cases of methyl-nitrosourea-induced cataract in rats undergoing ovariectomy (Guthrie et al., 2004). Studies show that estrogen contribution to reducing the development of cataract is a result of a protective effect on the receptor-mediated processes. This is because the reverse-polymerase transcription chain reaction shows expression of  $\beta$ - and  $\alpha$ -type estrogen receptors by the lens cells (Taskinen, 1998).

Other studies have hypothesized that estrogen confers protection against the development of eye cataract by converting growth factor  $\beta$  (Hales et al., 1994; Hales et al., 1997). It is described that, findings from cultured rat lenses, transforming growth factor  $\beta$  induces opacities beyond those



present in the eye. While *in vitro* or *in vivo* estrogen-based hormone therapies restore eye resistance, the lenses from rats exposed to ovariectomy have been reported to show damages resulting from the transforming growth factor  $\beta$  (Bigsby, et al., 1999). Finally, some research findings propose that estrogen show antioxidant effects in HT treatment that has beneficial impacts in the development of cataracts (Cumming and Mitchell, 1997; Niki and Nakano, 1990).

Most of the current claims rely on the Framingham prospective study design (Leske et al., 1991). Furthermore, pathology reports and surgical notes from the study have been useful for assessing and substantiating menopausal status. For instance, postmenopausal women might be aware of their hysterectomy, though it is less common that they are aware they underwent bilateral oophorectomy (Caulfield et al., 1999). The gathering of information regarding the duration of HT use is essential, as this data allows researchers to evaluate the long-term impact of the therapy on the eye conditions (Ettinger and Friedman, 1996).

Ettinger and Friedman (1996) and Henderson et al. (1991) report a reduction in the mortality of women who undergo prolonged use of estrogen in comparison with women who were subjected to shorter durations of HT use. Even so, some scholars, such as Stampfer et al. (1991), fail to concur that a shorter duration of HT use is more effective in reducing DES in postmenopausal women (Stampfer et al., 1991). Moreover, women that have used HT for a period of 7 years exhibit a suggestively high bone mineral density compared to patients that were administered with estrogen for shorter durations (Keil et al., 2007). Estrogen was collected independently in both the Framingham eye study and in the Framingham heart study. This approach ensured that the problem of partial bias was eliminated in ascertaining the outcome status and the exposure to HT use.

### **3.2.5 Hormone therapy and Ocular Surface**

For most postmenopausal women, relief of the dry-eye syndrome is achieved today by restoring premenopausal levels of sex hormones (Keating et al., 1999). Even if several benefits of the use of HT have been identified (Belchetz, 1994), increasing cases of adverse events are also being reported (Tavani and La Vecchia, 1999). That HT impacts the ocular surface in dry-eye syndrome appears to lack consensus in the current literature. The positive impact through which HT restores the tear functions can be explained by the existence of progesterone and estrogen receptors. However, at the moment, research findings have not explained the specific roles that

these receptors play, either in promoting the positive tear functions or in propagating negative impacts on the tear function.

Reports of relief from ocular discomforts are more numerous in patients that have been treated with HT than in an age-matched postmenopausal woman that have not received hormone therapy (Mathers et al., 1998; Mathers et al., 2002; Lang et al., 2002). However, the impact of the problems that come with identifying the co-causal role of age in hormone therapy efficacy must be emphasized. The decline of the significant ocular surface parameters—like stability and tear production—has been reported in postmenopausal patients. Administering HT has been reported to recover normal ranges of Schirmer test I value (Guaschino et al., 2003; Affinito et al., 2003; Altintas et al., 2004). Some authors, however, have not reported any role of HT in facilitating or limiting tear production for at least 90 days after hormone therapy, although there was observed improved tear production (Pelit et al., 2003).

Estrogen deficiency in postmenopausal women modifies the morphology of the conjunctival epithelium. This mechanism is realized through reduction of the density goblet cells that play a central role in the secretion of mucus. This process contributes to tear-film instability and eventual dryness. Hormone therapy appears to restore the number of goblet cells in increased tear production and in reducing the eye dryness (Pelit et al., 2003; Okon et al., 2001). As argued by Wied and Bibbo (2001), the conjunctival epithelium is subjected to a maturation process that is marked and regulated with hormonal fluctuation during menses. As predicted, the cycle develops and fades in postmenopause. Despite this, studies have found that this cycle can be restored with the use of estradiol treatment (Vavilis et al., 2007).

Based on clinical findings, it has been proposed that estrogen-based hormone therapy be administered in eye drops (Sator et al., 1998). Randomized trial data indicate a significant recovery of tear production and subjective symptoms in a group that underwent treatment with topical bestradiol, as compared with a group that received systematic-treatment after a 4-month treatment duration. The estradiol impact is associated with the stimulation of nitric oxide discharge in the blood. Such release can facilitate vasodilation of the lachrymal gland ducts in the eye (Sator et al., 1998; Brignole et al., 2008).

In this context, an epistemological study of 25,000 participants reviewed the actual efficacy of HT in improving the recovery of tear functions and improving dry-eye symptoms in postmenopausal women (Schaumberg et al., 2001). This study found that hormone therapy might

facilitate the production of dry-eye syndrome instead of alleviating it. To be specific, the use of estrogen alone as a treatment option is highly probable to result in the onset of dry-eye syndrome. To collect participant symptoms, the study used a questionnaire that has been criticized by some scholars for being subjective and for lacking specificity (Peterson et al., 2002; Barney, 2002). Nevertheless, an important observation has emerged from this large cohort research: caution should be exercised when prescribing hormone therapy for postmenopausal women with dry-eye syndrome (Altintas et al., 2004).

### **3.2.6 Hormone therapy and Eye Cataracts**

Initial studies suggest that men and women below the post-menopause age are about equally likely to develop eye cataracts (Kahn et al., 2007). However, during the menopausal age, the occurrence of cataracts increases in females more than in males of the same age-bracket (Sperduto and Hiller, 1984; Klein et al., 1992). The increased prevalence of eye cataract in postmenopausal women indicates a potential influence of estrogen in the retardation of cataract formation (McCarty et al., 1999). Previously, two cross-sectional and population-based studies have documented the valuable impacts of estrogen HT on specific eye cataracts (Klein et al., 1994; Cumming and Mitchell, 1997). Nevertheless, another cross-sectional study reports contrary findings. It argues that no protective effect can be established for hormone therapy (McCarty et al., 1999).

### **3.2.7 Hormone therapy and changes to the lachrymal secretion**

As far as the impact of hormone therapy on lachrymal functions is concerned, there is almost a consensus in the literature based on clinical research that the use of estrogen enhances quality and quantity of tears. Some researchers also agree that this improvement is significant statistically. For example, Altintas et al. (2004) report enhanced quantity and quality of tear function in persons using estrogen-only HT, compared to the persons not using the therapy for a period of 2 months. Another randomized control study by Affinito et al. (2003) indicated potential improvement in the secretion from the lachrymal gland in 25 participants who used progesterone and estrogen for 3 months, associated to the control group.

In a similar study, Fini (2009) reported no difference in a Schimmer's test and tear-film breakup time (TFBUT) test in 17 postmenopausal women who used estrogen for 3 months. Similarly,

Kumari et al. (2005) reported no significant alteration in foreign body perception, burning, and tearing after duration of six months of using progesterone and estrogen. In line with a randomized study undertaken by Belchetz (1994), the 70 women who used progesterone and estrogen combination for a period of six months indicated improved TFBUT and Schimmer's tests over those who used the placebo. These studies indicate that a combination of HT (progesterone and estrogen) reduces meibomian gland inflammation. Besides, the synthesis of the oily tears has resulted in a significant decline of chalazion in the eyelids as elaborated from a recent clinical study (Henderson and Madden, 2013). A *chalazion* is a meibomian gland cyst: a chronic infection triggered by a blockage of gland pores that results in the accumulation of tears.

The lacrimal gland produces the tear hyperoxidase which acts as antimicrobial and antioxidant enzymes to protect the ocular surface. The activity of this enzyme during menses exhibits cyclical changes and is positively associated with estrogen levels in the plasma. During menopause, however, the enzyme activity significantly reduces as a result of reduced estrogen production. Potentially, these changes may be positively correlated with dry-eye syndrome in menopause women. HT with progesterone/estrogen combination or with estrogen alone in postmenopausal women works to increase the activity of this enzyme. Due to increased activity of the tear hyperoxidase, HT was incorrectly linked to dry-eye syndrome before it became clear that HT can reduce problems of the ocular surface complication in postmenopausal women.

### **3.2.8 Hormone therapy and changes in conjunctiva and cornea**

Concerning the changes to the conjunctival epithelium, there impacts of HT are still controversial. Some reports indicate that there is an increase in the goblet cell density that contributes to an increased secretion of the mucous layer of the tears (Belchetz, 1994), while other literature findings have not established similar results (Avis et al., 2010) thereby warranting the need for further explanation. Pelit et al. (2003) have reported significant alterations in conjunctival epithelium in 17 postmenopausal women that used HT for 3 months. In addition, there was also an increase in the goblet cell density in addition to the fact that there were reduced symptoms of dry-eye syndrome in these women. Following a 6 month period of tibolone use, there is no difference in conjunctival cells compared to women who received progesterone and estrogen or those who received HT treatment (Taner et al., 2004).

Kuscu et al. (2003), who utilized progesterone and estrogen in postmenopausal women for 6 months, found no significant change in the degree of laxity or conjunctival vascular congestion. Hormone therapy does influence the corneal epithelium, but it is not statistically significant. A randomized controlled study of 25 menopausal women found that using progesterone and estrogen for between three and six months period results into the increased corneal thickness (Affinito, et al., 2003). Moreover, concerning the desquamation of the cornea, the use of progesterone and estrogen does not change in rate (Kuscu et al. 2003).

### **3.2.9 Hormone therapy and development and progress of cataract**

Menopausal women have been reported to have high rate of degenerating nuclear, while women that have shown late menopause indicate reduced risk of nuclear progression. These findings were reported by the Salisbury eye study, where a total of 1500 women above 65 years were studied for a period of two years (Freeman et al., 2004). It found no significant relationship between the incidences of cataract, total testosterone, sex hormone binding globulin, and levels of estrogen. The researchers showed a declined risk of cataract in women who had bilateral oophorectomies and received hormone therapy, and in patients who expressed excessive amounts of dehydroepiandrosterone sulfate (DHEAS). DHEAS is related to the protection of lens opacities and also has antioxidant properties (Defay et al., 2003). Nevertheless, according to the Salisbury study of Freeman et al. (2004), hormone therapy does not protect from the progress or incidence of cataract. Moreover, according to the POLA study of Defay et al. (2003), prolonged HT use seems to increase the problem of cataract development.

### **3.2.10 Hormone therapy and changes in intraocular pressure**

As discussed earlier, estrogen can affect the intraocular pressure (Altıntaş et al., 2004). Past literature findings have reported that there exists in association between female hormones and intraocular pressure or reduced hormone secretion during post-menopause. Studies have reported significant changes in the intraocular pressure in different menstrual phases (Guttridge, 1994; Avasthi and Luthra, 1967), and the successful administration of C-21 and C-18 steroids in glaucoma patients (Barney, 2002), showing a potential correlation between sex steroids and intraocular pressure. Nonetheless, there is little information that narrates on whether the intraocular pressure is affected by the hormone therapy.

In the early 20<sup>th</sup> century, the association between gonadal functions and intraocular pressure was documented by Imre (1921). Following succeeding research, the association between the menstrual cycle and intraocular pressure, and the impact of pregnancy and some hormone medications on intraocular pressure was studied (Green et al., 1984). Another study by Sano (1973) reported that there is an increase in intraocular pressure during pre-menstrual phase and a decline in intraocular pressure during ovulation. Ziai et al. (1994) have observed a decline in intraocular pressure and increase in the aqueous outflow during pregnancy as a result of increased progesterone production. The same results have also been documented during the luteal phase of the menstrual cycle.

The results indicate that progesterone can reduce intraocular pressure in menopause women, mainly during the start of the ovarian insufficiency in menopause cases triggered by progesterone. In a clinical research on 25 postmenopausal women, Altintas et al. (2004) have reported similar results during menopause where there is increased intraocular pressure. As such, the use of HT for a period of 2 months appears to decline the intraocular pressure from the research finding reported by the same study. Moreover, in line with the randomized-control clinical study by Affinito et al. (2003), there is a significant reduction in intraocular pressure after 3 months of progesterone and estrogen therapy on 25 post-menopause women, compared to the control group.

### **3.2.11 . Hormone therapy and the risk for glaucoma**

Reduced estrogen levels can contribute to increased symptoms displayed by the open angle glaucoma. A study by the Blue Mountains eye study evaluated a total of 2000 women aged 50 years and above for 2 years. This study found that there is a double risk of developing glaucoma when the menarche commences at the age of 13 over those who start earlier. The same study found that, so far as the menopause age is concerned, it has little significant relationship with glaucoma. On the other hand, the number of deliveries are related directly to open angle glaucoma, although there is declined prevalence of ocular hypertension (Lee et al., 2003). An additional finding by Lee et al. (2003) shows that HT reduces the risk of glaucoma, although not by statistically significant margins. This shows that estrogen has protective effect on glaucoma and reduces the risk of vascular resistance. Moreover, estrogen works to bind to the retina

receptors, resulting in the generation of nitric oxide. The oxide work to dilate the blood vessels and to facilitate aqueous humor flow from trabecular mesh-work. Mechanisms are currently considered those through which estrogen protects against the risk of glaucoma (Lee et al., 2003).

### **3.2.12. Hormone therapy and changes in ocular blood flow.**

Various measurements show that estrogen levels in serum contribute to increase in ocular blood flow. Some of the tests by Altintas et al. (2004) indicate that the level of, resistance index, diastolic velocity, and peak-systolic volume are all affected by estrogen levels in serum. A comparison study of 25 women of same age that still had menses indicates that, during menopause, the resistance index increases in both the central retinal artery. Postmenopausal women show increased resistance index, and the resistance index decreases at the end-diastolic velocity compared to pre-menopause women (Toker et al., 2003).

However, there is increasing controversy on whether HT has any impact on ocular blood flow. Altintas et al. (2004) performed a clinical study in which, after 2 months of HT use in postmenopausal women. He showed a reduction in resistance index and flow at the end-diastolic velocity in the central retinal artery (Altintas et al., 2004). Women who use HT with estrogen have a 40% increased risk of arteriolar narrowing, according to a clinical trial that recruited 1900 women. In a similar research, Leung et al. (2004) recruited women above 65 years and found that HT use does not exert potential benefits on the retinal arterioles of postmenopausal women. Therefore, as in other topics related to the impact of HT on propagating dry-eye syndrome in postmenopausal women, controversy remains about the efficacy of hormone therapy in improving the ocular blood flow.

## **3.3 Menopause and hormone changes in menopause and post menopause women**

### **3.3.1 . Hormone Changes during menopause and Eye Condition**

Menopause and its corresponding intermediate phases influence the psychological and physical well-being of women. This assertion is supported by decades of research that show how hormonal changes influence the experience of menopause. A number of studies on the effects and symptoms of menopause document adverse effects on women's occupational performance (Burton et al., 2004) and physical performance (Sowers et al., 2001). Other studies report reduced health and wellbeing (Kumari et al., 2005) accompanied by chronic medical conditions such as osteoporosis (Guthrie et al., 2000; Guthrie et al., 2004) and cardiovascular disease (Carels et al., 2004; Pérez-López, et al., 2009).

Besides the physical impacts of menopause, women at this stage are also subjected to psychological effects such as prolonged depression (Turner, et al., 2004; Timur and Şahin, 2010; Llana, et al., 2012) and forfeiture of quality of life (Li, et al., 2000; Jacobs, et al., 2013; Laferrere, et al., 2002; Avis, et al., 2010). Of more concern to gynecologists and obstetricians is the increased prevalence of dry-eye syndrome linked to hormonal impacts on the ocular surface. The correlation between hormone change during menopause and dry-eye syndrome is reported by several scholars (such as Versura and Campos, 2005; Schaumberg, et al., 2003).

Characterized by changes in estrogen and progesterone balance, postmenopausal women have been reported to experience blurred vision, photophobia, pain, stinging sensations, foreign-body sensations, and ocular burning (Pong, 2013). With subsequent intermediate phases, the resulting impact of hormonal changes exposes women to pain, red eyes, and eventual fatigue (Henderson and Madden, 2013). Given the diverse range of disease symptoms, the identification of dry-eye

Syndrome as a hormonal ocular condition can be validated (Henderson and Madden, 2013). Therefore, the detrimental impact of hormonal changes in women's eye health (Schiffman, et al., 2000; Nichols, et al., 2002; Mertzanis, et al., 2005; Friedman, 2010) and normal vision



(Miljanovic et al., 2007) must be established to outline potential ways to improve care and therapeutic intervention in postmenopausal women.

The exact role of hormonal imbalance in eye conditions of postmenopausal women is still a subject of debate. Some scholars attribute dry-eye to a combination of sex-hormone and environmental changes (Schiffman, et al., 2000). Other scholars attribute dry-eye syndrome to changes in the endocrine systems of women (Baudouin, 2001; Ogueta, et al., 1999; Munaut, et al., 2001; Sullivan, et al., 2002; Sullivan, et al., 2012; Sullivan, et al., 1999), especially in postmenopausal women (Mathers, et al., 1998). Sullivan et al. (1999) argue that the condition is worsened in postmenopausal women who subject themselves to various therapeutic options to restore normal balance in their daily lives (Schein et al., 2007). Hormone therapy (HT) is among the many therapeutic options available for alleviating dry-eye syndrome in postmenopausal women.

### **3.3.2 Hormone Changes and Dry-Eye Syndrome during menopause**

During menopause, the ocular surface of most women becomes drier. The dryness is attributed to a reduced quality or quantity of the pre-ocular tears present in the film. Schaumberg et al. (2003) stated that the risk of DES, in both men and women, is similar and can occur at any age. However, the incidence of dry eye appears to increase as patients get older, and women are more likely to develop the syndrome than men. Some researcher, such as Warren (2009) and Schein et al. (2007), argue that there is no substantial variance between women and men in the incidence of dry-eye syndrome. Despite continued debate on the topic, several research using large epidemiology studies in older patients report that women are more likely, compared to men, to report dry-eye symptoms (Chia et al., 2013; Lin et al., 2003).

## **3.4 Hormone therapy and eye disease in Postmenopausal Women**

### **3.4.1 Effects of HT on eye conditions of Postmenopausal Women**

Chan et al. (2006) regard HT as a regular administration of progesterone and estrogen in postmenopausal women with an aim of minimizing the physical and psychological effects of menopause. With time, HT has gained support as a suitable solution to menopause-related complaints (Hulley et al., 1998). However, other studies (Vavilis et al., 2007; Jensen et al., 2010) showed contradictory results.

Literature on the relationship between HT and DES in postmenopausal women reflects both the advantages and risks of HT use. Therefore, the relationship between HT, menopause and eye-disease conditions continues to be a subject of heated debate. For example, the use of HT changed abruptly when large clinical trials reported that the treatment poses more health risks than benefits. As concern about the relationship between dry-eye syndrome and hormone therapy increase, some quotas of clinical care have reduced or stopped using HT for postmenopausal women (Hodges and Dartt, 2003).

Hormone therapy that involves estrogen combined with a progesterone substrate appears to worsen eye disease by making the eyes denser. In addition, when HT is followed for a prolonged period of time, it appears to increase the risk of eye conditions. Therefore, the risks of hormone therapy and its correlation with eye conditions depend on whether estrogen is administered individually or combined with progestin. The risk of DES in postmenopausal women also depends on the age of menopause onset and the dose type of estrogen (Wied and Bibbo, 2001).

Initially, researchers assumed that HT reduces the occurrence of dry eye in postmenopausal women (Warren, 2009; Turner, et al., 2004). Some studies have sought to establish the correlation between the two. For example, in a study of hormone therapy in Asian women, Shaharudin et al. (2008) reported that women who receive combined progesterone and estrogen therapy are more likely to develop dry eye (84.2%) than women who receive estrogen alone (45.5%) ( $p=0.042$ ). The finding indicates that the tear film is less stable in women who take estrogen and progesterone HT than in menopausal women who take estrogen therapy alone. However, as argued by other authors such as Schaumberg (2006) and Sullivan et al. (2012) fails to consider the protective effect of HT in DES.

One possible explanation for this lack of correlation is that most researchers base their conclusions on the patient's symptoms and the self-reported clinical diagnosis. In the study by Shaharudin et al. (2008) all the patients were subjected to an ocular exam for dry-eye syndrome by the same clinician. Such an approach reduces selection bias and standardizes the dry-eye diagnosis criteria. The lack of relationship between HT and reduced dry-eye symptoms may also be attributed to the genetic makeup of Caucasians and Asian populations (Shaharudin et al., 2008).

Using a population-based research Lin (2011) attempted to evaluate DES in elderly women and found that dry-eye syndrome is more prevalent in the Chinese population than in the Caucasian population (11 women used estrogen hormone in the control group, whereas 19 used estrogen and progestin pills in the test population) These findings indicate that DES is more prevalent in women who use an estrogen-progestin combination ( $p=0.003$ ) than in women who use HT with estrogen alone (Lin, 2011).

Other studies show that the risk of eye-cataract formation in postmenopausal women who use hormone therapy appears to diminish with a prolonged use of estrogen therapy. One such claim has been made by the Framingham Heart Study of postmenopausal women who used estrogen for a period of ten years or longer. The findings show that risk of lens opacities is reduced by 60% compared to the controls (Worzala et al., 2001). In an adjusted research model, Altintas et al. (2004) showed that extended estrogen-hormone therapy in postmenopausal women is inversely related with the presence of nuclear lens opacity. Women who used HT for 10 years had a significant reduction in the risk of eye disease (odds ratio, 0.4; 95% compared to non-HT users with a confidence interval (0.2-1.01).

It appears that extended use of estrogen in HT is linked to reduced posterior sub-capsular opacity at a marginal level of significance. However, no association has been made with the cortical opacities. In patients that has received surgical menopause, the risk of developing posterior sub-capsular opacities appears to increase significantly compared to that of women who are experiencing natural menopause. Besides, no correlation has been found between the age of menopause and lens opacities (Worzala et al., 2001). In another study, Freeman et al (2001) showed that postmenopausal women who use HT have a 70% to 80% reduction in sub-capsular and nuclear opacities.

Freeman et al. (2001) asked whether hormone therapy is related to the prevalence of different lens opacities after controlling for androgen-estrogen exposure. Their study maintains that both recent and current HT usage protects against nuclear opacity, and that hormone-therapy use protects against dry-eye condition autonomous of endogenous estrogen use. These findings illustrate that the risk of estrogen exposure, including increased numbers of births and birth control pills, are related to a low prevalence of nuclear opacity (Freeman et al., 2001). These findings are consistent with the findings of the Beaver-Dam eye study, which was undertaken to

show the association between nuclear opacity and the use of hormone therapy (Jensen et al., 2010).

The Beaver Dam eye study (2010) failed to find any statistical relation between the current use of HT in postmenopausal women and the development of eye syndrome. This is despite the fact that the odds ratio is in the protective direction in older postmenopausal women. It also found low rates of severe nuclear opaqueness in young females that were using family planning pills (Klein, et al., 1994). However, Freeman et al. (2001) criticizes the findings from Blue Mountains eye study (1997) that documented low rate of cortical opaqueness when the HT is used, high rates of posterior subscapular cataract opacity when HT is used, and no relationship with nuclear opacity (Cumming and Mitchell, 1997). The explanation for these variations is not obvious, although the systematic valuation of lens dullness from Beaver Dam eye research was the same to the one used by Blue Mountains eye study, and though the results of the Blue Mountains study are similar to those of Freeman et al. (2001). Variations in the grading systems used to measure lens opacity are the likely explanation for the observed differences in the above studies.

Nonetheless, other studies continue to report the relationship between HT and dry-eye syndrome. A study by Schaumberg et al. (2001) sought to determine the correlation between dry-eye and hormone therapy. These women (n=665) were provided with baseline information about hormone therapy and were checked at 12 months, 24 months and 36 months for dry-eye. The results showed that postmenopausal women that use hormone therapy—specifically estrogen-based therapy only—show elevated risk of DES. Therefore, clinicians who care for postmenopausal women who are considering HT therapy should be examined and informed about the potential side-effects and future complications of the treatment (Schaumberg et al. 2001).

It appears that a postmenopausal woman that uses hormone therapy have an increased risk of eye disease compared to non-users (estrogen and progestin/progesterone, 29%; estrogen alone 69%) (Smith et al., 2004). When age and other factors were adjusted for, postmenopausal women who have never used hormone therapy reported no negative signs of the dry-eye condition (Schaumberg et al. 2001). The relationship between dry-eye syndrome and HT was found to be consistent with all the definitions of the dry-eye condition used in the study, and it remained the same for the clinical population diagnosed after the start of the hormone therapy. Notably, the

risk of dry-eye syndrome seemed to increase in the examined population if the therapy went on for many years (Schaumberg et al., 2001).

Worzola et al. (2001) showed that when postmenopausal women use estrogen, there is a marked risk reduction in lens opacities (Worzala et al., 2001). Basic studies indicate that the level of the sex hormones can influence the operation of both the meibomian and lachrymal glands. Preliminary clinical and laboratory studies indicate that, while there is a beneficial relationship between androgens and their effects on meibomian and lacrimal gland functions, estrogen can play a role in increasing the risk of dry-eye syndrome (Avis et al., 2010). Given these observations, additional study is needed to determine the impact of estrogen on meibomian gland, which is composed of estrogen receptors (Barney, 2002). As such, the benefits of using progestin/progesterone combination therapy over estrogen-only therapy to treat dry-eye condition need to be investigated in future studies.

### **3.4.2 Effects of HT on Eye Sensitivity in Postmenopausal Women**

Change in hormone profile has been reported to increase the risk tear function and ocular disease (Sullivan et al., 2002; Mamalis and Harrison, 1996). The relationship between tear production and hormone levels affect the sensitivity of an eye to its surrounding environment. Whether this correlation is linked directly to the lachrymal and also in the meibomian gland is so far not known. The impairment of the control of lachrymal secretion plays a significant part in disease development in postmenopausal women. Mathers et al. (1998) report a dissimilar association between tear production and the levels of hormone in pre-menopausal and post-menopausal patients. Besides, significant levels of hormones in the serum is negatively associated with tear production, whereas the estrogen levels are positively correlated. In contrast, the correlations in postmenopausal women are reversed, with higher levels of testosterone linked to increased tear function. High levels of testosterone are associated with reduced tear function (Mathers et al., 1998). The levels of testosterone stayed within the normal range during the course of the study.

Sullivan et al. (1990) undertook a case control study and found that Sjogren's syndrome is not linked alteration of serum levels of estrogen, androstenedione, testosterone, or transdermal  $17\beta$ -estradiol. They find that androgen-deficiency is related to Sjogren's syndrome. At the beginning, the levels of hormones were similar in both control and study groups. As a result, the authors did not explain the presence of DES in patients with therapy. In such group of women,

nearly all androgens and all estrogens are locally synthesized in the peripheral target tissues. Production occurs in the inactive adrenal steroid precursor dehydroepiandrosterone (DHEA). This means that little contribution is made from the human sex-steroid hormones, principally those that initiate the secretion of testosterone. Menopause duration was found to be significant in persons with or without the dry-eye syndrome. The authors argue that such an abnormal observation can be clarified by the reduced excretion of the DHEA by the adrenal organ of the tissue specific steroidogenic enzymes in the eye periphery. This may be another risk in the dry-eye disease of postmenopausal women.

Previous studies have reported that eye sensitivity decreases under prolonged medication in estrogen-based hormone therapy. Even if reduced sensitivity is common in postmenopausal women, Guaschino et al. (2010) show that use of hormone therapy significantly improves sensitivity of the eye. A recent cohort study assessed the correlation between dry-eye syndrome and hormone therapy. It reports that postmenopausal women who receive HT exhibit an amplified risk of delicate eye by 30-70%, considering the HT formulation administered. In addition, risk in eye sensitivity appears to increase with the duration of HT administration. For every 24 months of hormone therapy, the risk rose by 15%. In new users, there appears to be a significant surge in risk (5.7%). The rate is highest in women who use estrogen alone (9.1%). There is a risk of 6.7% in persons who use a combination of estrogen and progesterone therapies. This indicator shows that progesterone has a protective effect on a sensitive eye. In this study, all patients who used HT suffered adverse events—especially women in a control group who did not have any dry-eye syndrome at the start of the study. A continuous estrogen therapy—estradiol transdermal estrogen therapy—has been documented to trigger ocular idiosyncratic reactions and keratitis sicca (Avis et al., 2010). Moreover, the use of HT in postmenopausal women may further complicate the ophthalmic techniques by activating a sensitive eye that degenerates to a dry-eye syndrome or to some other related tissue-specific ocular change. All of these controversies can be resolved via long-term follow-ups of controlled clinical trials.

As noted in the previous sections, different studies have provided conflicting reports over the years on the association between HT, menopause, and various conditions of the ocular surface. One large-scale study is the Framingham study. In this study, Worzala et al. (2001) documents the fact that hormone therapy has an advantageous effect on lens opacities. Additional

observations maintain the strength of this correlation, the biological plausibility of the correlation, the uniformity of the finding from epidemiological studies, and the dose-response type of the correlation. All of these correlations suggest that HT is significant in protecting the ocular region. Therefore, the outcome of this study indicates a potential supplementary benefit in using estrogen-based therapies to manage DES in women.

Results from Framingham eye research indicate that, prolonged use of estrogen increases results in decreased risk of developing nuclear opacity. In addition, the cases of posterior opacity reduced among estrogen users, although the results were just borderline. On the other hand, the same patients who used estrogen-based HT failed to show any signs of improved cortical opacities. The argument that estrogen-based hormone therapy has a protective effect on lens opacity, according to the research, is elucidated by an observation that most of the patients with opacity showed posterior or nuclear sub-capsular opacity. Although some scholars dispute these findings, the study argues that different lens opacities are affected differently by HT during dry-eye syndrome.

Additional epidemiology studies have reported a possible relationship between lens opacities and estrogen. For instance, the Beaver Dam eye study documented a reduced risk of nuclear sclerosis in postmenopausal women who use estrogen-based HT (Klein et al., 1994). Both older-age and younger-age menopause phases are associated with declining levels of lens opacities. This finding also indicates that hormone therapies influence the process of cataract formation. However, no results have been found to suggest an association between HT and posterior sub-capsular opacities or cortical opacities (Klein et al., 1994).

The Melton eye research documented that increased treatment using oral contraceptives contributes to fewer cases of nuclear opalescence, although this association is not linked to any effects on the cortical opacity. Inconsistencies reported by these studies have been attributed to the various definitions of hormone use strengths and opacity. This approach affects the relations between nuclear opacity and estrogen use. Nonetheless, two studies report dissimilar results on possible relation that exists between HT use, postmenopausal women and dry-eye syndrome.

According to Blue Mountains eye research, 65-year-old patients that used estrogen showed reduced cases of developing cortical lens opacity compared to the ones that did not use estrogen-based HT (Cumming and Mitchell, 1997). The study shows that women who use HT and are

natural postmenopausal have an increased incidence of posterior sub-capsular opacity (Cumming and Mitchell, 1997). However, a similar study by the Melbourne Visual Impairment Project failed to establish any correlation between eye cataracts and hormone therapy (McCarty et al., 1999).

### **3.4.3 Effects of HT on the ocular-surface homeostasis in postmenopausal women**

Over the last four decades, a number of research findings have documented a possible correlation between HT and ocular homeostasis in postmenopausal women (Barney, 2002; Altintas et al., 2004; Affinito et al., 2003). This homeostatic function has been linked to the role played by estrogen and androgen receptors positioned on the conjunctival and also on both the corneal epithelium and Meibomian glands. Barney (2002) points out that the ocular surface is often an integrated segment. In the event of any dysfunctions in hormone balance from HT, this will result in unstable and scarce pre-ocular tear film and accompanying dry-eye syndrome.

According to Burton et al. (2004) HT in postmenopausal women is particularly likely to contribute to unstable surface homeostasis over time, resulting in a chronic inflammatory condition of the dry eye. Older populations of female patients are particularly prone to changes in ocular-surface homeostasis (Burton et al., 2004). Although endocrine changes in postmenopausal women increase the negative impact of dry eye, it is still controversial whether HT can compensate for androgen or estrogen deficiency (Brignole et al., 2008). Besides, the exact mechanism of this hormonal imbalance and how it impairs the ocular surface function in postmenopausal women is still debated.

Another debatable issue is in relation to the efficacy of HT in the possible enhancement of the ocular-surface homeostasis in the recovery of the tear function and reduction of the dry-eye symptoms (Fini, 2009). This controversy is elevated by findings from the scientific literature that seems to contradict therapeutic findings concerning the benefits of HT in the amelioration of the ocular surface (Fin, 2009). The therapy commonly used to decrease symptoms of ocular homeostasis involves topical administration of tear substitutes. Schaumberg et al. (2002) state that HT can have functions, as it is based on androgen- or estrogen-based eye droplets. HT therefore represents an innovative and promising treatment that is based on significant empirical



scientific rationale (Schaumberg et al., 2002). Nevertheless, the risk of ocular-surface-homeostasis imbalance increases with age along with a substantial decline in the production of tears.

The rate of ocular surface impairment can also become elevated during menopause as a result of increased hormonal imbalance. Even so, it is not known whether HT facilitates and worsens this state (Hodges and Dartt, 2003). The current body of literature has been yet to determine whether changes to the ocular-surface homeostasis are related to excessive estrogen or to its deficiency.

More research also is needed in order to determine whether this problem is related to imbalances in androgen insufficiency or due to imbalances in androgen/estrogen secretion. The effect that these hormones have on the ocular surface among postmenopausal women continues to be contested, as is the influence of HT on postmenopausal women (Tomany et al., 2004; Li et al., 2011).

#### **3.4.4 Effect of HT on ocular hemodynamic in postmenopausal women**

Atilla et al. (2001) sought to examine the impacts of HT on the ocular hemodynamic in postmenopausal women. A total of 20 postmenopausal women underwent ocular Doppler ultrasonography before they were put on HT treatment. A control group of 20 women was also selected for the study, but never underwent the treatment. Radiologists blinded to the study were used to measure vascular resistance, flow velocity at the central retinal artery, posterior ciliary artery, ophthalmic artery on the participants. The subjects did not report ocular or systemic diseases or any history of medication. Findings from this study reveal that HT is significant for relieving cardio-protection, vasomotor symptoms, and preventing osteoporosis in postmenopausal women. Despite vaso-occlusive complication being reported, HT in this study was not found to change the ocular hemodynamics as detected by the Doppler ultrasonography.

Substantial gender centered diversity in the occurrence of age-related eye hemodynamic changes indicates the impacts of HT on exacerbating dry-eye syndrome. Changes to the nuclear cataract, high intraocular pressure, keratoconjunctivitis sicca, posterior vitreous detachment, idiopathic macular hole, primary open-angle glaucoma, and macular degeneration raise the possibility that use of estrogens during HT may result in direct hemodynamic alteration of the eye (Klein et al., 1994; Evans et al., 1998). Moreover, elderly women who are undergoing hormone therapy show reduced cases of nuclear cataract (Klein et al., 1994; Cumming and Mitchell, 1997), age

associated macular breakdown (Smith et al., 1997), and development of glaucoma (Sator et al., 1997). Increased evidence continues to show an HT interruption to the ocular hemodynamics and increased risk for a number of major ocular diseases, such as glaucoma, which has been linked to ocular ischemia (Hulsman et al., 2001; Green et al., 1984).

The effect of HT on the blood flow in the ocular area has been studied by Atalay et al. (2005). To determine the impact of HT on eye vasculature and ocular hemodynamics, the authors researched more about the small ocular artery. Both the peak and the end diastolic velocities were measured during the study. They also recorded pulsatility and resistive indexes. The two indexes are important for measuring the impedance of the blood vessel might have been difficult to examine using the velocity measurements alone. They reported that, in the study group receiving HT, a significant reduction in the pulsatility index was reported in the central retinal artery after treatment, as the control group indicated an increase in pulsatility index values after treatment (Atalay et al., 2005). These research findings reflect previous studies by (Belfort et al., 1995). Luckas et al. (1998) also report that the use of progestin-rich HT in postmenopausal women results in a significant increase in the carotid artery pulsatility index. However, there was no increase in the ophthalmic artery Doppler measurement.

### **3.4.5 Effect of HT on intraocular pressure in Postmenopausal women undergoing**

For the eye to serve its normal functions as a transducer and light-gathering organ, it is essential that intraocular pressure be maintained within normal limits. Intraocular pressure in the eye results from an intricate balance between the drainage of the aqueous humor and its formation (Affinito, et al., 2003). McCarty et al. (1998) report an increase of intraocular pressure in patients under HT treatment that results in defects in the glaucomatous visual field. Even if increased levels of intraocular pressure are not a risk factor during the glaucomatous impairment of the optic nerve, the more likely occurrence is the progression of glaucomatous optic nerve breakdown is as a result of abnormally high intraocular pressure (McCarty et al., 1998).

Previous studies indicate that postmenopausal women under HT management record consistently high values of ocular-pressure impairment (Bron, 2001; Mathers et al., 1998). Most routine impairment studies have been limited to clinical populations of persons aged 40 years and above,

because this group is considered to be at a high risk for developing glaucoma (Guaschino et al., 2003). Intraocular pressure can be influenced by variations in sex hormones during menopause, resulting in increased risk of developing glaucoma. The normal range of intraocular pressure is maintained at 10 mmHg to 20 mmHg. When the pressure ranges between 21 mmHg to 24 mmHg, the values are deemed suspicious. Pressures above 24 mm Hg are considered abnormal (Lang et al., 2002).

Abelson et al. (2000) report a higher intraocular pressure in postmenopausal women than in premenopausal women. One significant difference between postmenopausal women and menstruating women is the variation in the hormone levels. The postmenopausal phase is characterized by very low levels of progesterone and estrogen compared to the premenopausal phase. Given this decline in progesterone and estrogen levels, it is probable that menopause does play an important role in altering the various components that regulate the intraocular pressure mechanisms (Abelson et al., 2000; Barney, 2002). Currently, there is a consensus that estradiol increases the levels of enzyme nitric oxide in the endothelial activities (Becquet et al., 1997). As a result, estradiol increases the prostacyclin and nitric oxide vasodilation while reducing the capacity of the musculature to respond to endothelin 1 (Schechter et al., 2002).

Moreover, there is sufficient evidence to indicate that progesterone possesses properties similar to glucocorticoid antagonism. Today, glucocorticoids have been reported to play a significant role in elevating the intraocular pressure in postmenopausal women (Weinreb et al., 1981; Garbe et al., 1997). As a result, progesterone may reduce the risk of exposure to hypertensive impacts of the endogenous glucocorticoids in a process of competing for receptor binding sites. These receptors are commonly localized in the network of the human trabecular cells that can bind both progesterone and glucocorticoids. With low levels of progesterone in postmenopausal women, the reduced intraocular pressure effect may be lost. This may result in high intraocular pressure. Moreover, intraocular pressure has been shown to be reduced with prolonged administration of hormone therapy (Sator et al., 1998).

A study done in a Korean population observed that the mean IOP increases proportionally with the degree of obesity in both males and females (Lee et al., 2002). Similar findings were also reported by Barbados and the Beaver Dam eye studies (Wu and Leske, 1997; Klein, et al., 1992). Danish Zafar et al., also observed a positive correlation between BMI and IOP in both males and

females among a Pakistani population (Zafar et al., 2010). The association between intraocular pressure and body-mass index has been reported to be significant in postmenopausal women. The reason for this correlation may be attributed to the dual impact of body-mass index and declined sex-hormone secretion. Wu and Leske (1997) suggest that orbital pressure resulting from fat causes an increase in pressure on the episcleral veins and also reduced pressure in the outflow (Wu and Leske, 1997). Bulpitt et al. (1975) pointed out that obesity may increase the viscosity of blood through by increased hematocrit, hemoglobin, and red-blood-cell count, thereby increasing the resistance and outflow of the episcleral vein (Bulpitt et al., 1975).

### **3.4.6 Effect of HT on ocular tear among postmenopausal women**

Other studies have evaluated the impact of HT on various dry-eye symptoms, such as ocular tear among postmenopausal women. Some of the symptoms assessed include corneal thickness, intraocular pressure, lachrymal secretion, and complaints about climatic ocular conditions. 50 women were subjected to a randomized control study for a period of one year after menopause. Group A consisted of 25 women who were given 50 µg/day of transdermal 17β-estradiol and the amount of medroxyprogesterone acetate administered was 10/mg/day. The other 25 women were untreated, forming the control group. All 50 women underwent an eye exam at the start of the study. After 3 to 6 months, they were assessed for ocular diseases, and measurements were taken of corneal thickness, intraocular pressure, and lachrymal secretion.

The study findings indicate that a significant deference existed between the study and the controls. After the 3-to-6-month evaluation period, researchers noted a significant decline in the total number of women that reported to have ocular problems. They also observed substantial decrease in disease severity than the results obtained for the controls ( $p < 0.01$ ). After 3 months, the secretion at the basal lamina was higher than the secretion recorded in the controls ( $P < 0.01$ ). Additionally, a significant reduction in the intraocular pressure was also recorded after 3 months into the test, and a slight but non-significant enlargement in corneal thickness in the test group at 3 and 6 months in contrast to their base values. These findings indicate that HT may have a beneficial impact on ocular symptoms. It may increase corneal thickness and lachrymal secretion, and may reduce intraocular pressure.

As discussed in the previous sections, sex hormones may influence ocular physiology. Undeniably, an upsurge on prevalence of ocular pathologies like arteriosclerosis obliterans,

arteriosclerosis obliterans, and glaucoma, and also the ocular symptoms, has been reported during different climacteric periods. Out of the 19 reported ophthalmic complaints of postmenopausal women recorded by Metka et al. (2010), 7 are linked to tear function. Tear function is the symptom most often associated with a feeling of dryness. Hence, impaired lachrymal secretion may be a central factor in the pathogenesis of postmenopausal ophthalmic symptoms.

Some of the diverse factors reported for the intraocular pressure can be caused by the administration of hormone therapy. Research findings suggest a significant decline in intraocular pressure during pregnancy and luteal phases. From this observation, it appears that progesterone serum levels do have an impact on this feature. It seems that when ovarian functions are interrupted, a negative impact on intraocular pressure results. An epidemiological data supports this hypothesis that shows an increase in age is connected with an increase in intraocular pressure, as evident in persons with 50 to 55 years. Additionally, glaucoma is less prevalent in women younger than 40 as compared to women over 50 years.

### **3.5 Contributions of the present study**

Several studies have examined the extent to which menopause is correlated with ocular pathological manifestations, and how HT can prevent these pathological changes from occurring. The international review finds that hormone changes during menopause largely affect the change in lachrymal secretion, the amount of tears, and alterations to the cornea and conjunctiva. Other potential eye changes that occur due to HT use include progress and development of cataract, change in intraocular pressure, and the flow of the ocular blood. However, in other patients, a reduced quality and also the quantity of the secreted tears further worsens the symptoms of eye dryness. In women who use HT, there is a continued controversy on the restorative function of HT treatment on the ocular surface in terms of enhancing increased secretion of quality and quantity of tears.

Critics have shown that postmenopausal women that receive HT show increased inflammation of the meibomian gland as a result of increased release of immunoglobulin A, lysozyme, and hyperoxidase secretion in the tears. The increased inflammation contradicts the supposedly medicinal functions of HT to reduce dry eye symptoms. On their part, proponents argue a possible positive impact of HT on DES as due to improved conjunctival epithelium and additional protection to the epithelium. However, HT use does not appear to prevent the

progression or development of cataract. This has raised questions concerning the possible effect of estrogen and other hormone imbalances on the ocular surface (Schaumberg et al., 2002).

Despite decades of speculation and research on the matter, the relationship of female sex hormones and DES continues to be elusive. The problem is further compounded by the complex association of dry-eye syndrome and both high and low estrogen states, which leads researchers to deliberate the impact of estrogen and androgen hormones as possible triggers of dry-eye disease (Sullivan, 2004; Schaumberg et al., 2001).

Although androgens can account for the majority of the gender variances reported from lacrimal tissue studies, the impact of estrogen is not clear. Sullivan (2004) indicates that the role of androgen hormone in post-menopausal women is often conflicting. At the moment, there is a consensus in the literature that reduced production of lipids and the size of sebaceous glands is affected by estrogen (Sullivan et al., 2002; Evans et al., 2012). Hence, it is possible that dry-eye syndrome is more severe in the event of high plasma estrogen—such as during hormonal contraceptive use or pregnancy. Furthermore, besides the complex influence of female sex hormones on dry-eye syndrome, another problem among the postmenopausal women is that wearing contact lens exacerbates the symptoms of DES. Epidemiological studies indicate that, across the globe, up to 80% of postmenopausal women who use HT present with clinical symptoms of dry-eye disease (Chalmers and Begley, 2006; Begley et al., 2002). In the era of hormonal contraceptive and hormone therapy use, some clinics have reported cases of intolerance in women under HT (Brennan and Efron, 1989; Ruben, 1966; De Vries, et al., 1978; Goldberg, 1970). Even among studies that have attempted to explain the relationship, there is always controversy and lack of consensus on how HT propagates or prevents dry-eye syndrome in postmenopausal women (Ruben, 1966; Goldberg, 1970 (Avis et al., 2010; Affinito et al., 2003). Today, there is increasing evidence that the use of tear osmolality testing can be helpful in understanding dry-eye syndrome. This is important given its significance as a global marker in cases of aqueous and evaporative tear-deficient subtypes of the dry-eye syndrome (Khanal, et al., 2008; Lemp, et al., 2011; Sullivan, et al., 2010).

Nonetheless, the exact relationship between hormone therapy and dry-eye syndrome is poorly understood. Given that a growing number of women at menopause receive HT to relieve their symptoms, the relations between HT and dry-eye syndrome should be examined. While HT

continues to gain popularity as a ministrations approach for menopause-related complaints, its potential threatens DES progression requires further investigation. This is because dry-eye syndrome can impair the normal functioning of affected patients. Therefore, a study that can validate the relationship between dry-eye and HT use during post-menopause is essential.

The objective of this thesis is to scrutinize the relationship between HT use and DES in postmenopausal women and to thereby provide women and concerned medical professionals with a balanced study of the effects of HT use from an ocular perspective. The findings will open avenues for future research to achieve two objectives: to develop more effective treatments related to menopause and/or to develop solutions to eliminate risks involved with the use of HT. The knowledge acquired from this study will add to the literature in obstetrics and gynecology regarding the correlation between HT and dry-eye syndrome

## 4. Materials and Methods

### Materials and Methods

**4.1- Materials:** The objective of the study (cross-sectional) was to establish an association between the use of HT among postmenopausal women and its impact on DES. The primary approach of this design is descriptive through the use of a questionnaire survey. Two measures of outcome were examined in the course of the 36 months. First, the severity of the symptom levels in the patients was measured using the OSDI index levels of severe, moderate, mild and normal. This OSDI index was used to identify the correlation between dry-eye syndrome and female sex hormones. Second, an assessment was made concerning the severity of the symptom levels among postmenopausal women under HT and women not under HT. This approach was meant to establish a correlation between DES and HT in postmenopausal women.

To confirm the presence of the DES in the selected sample, questions were asked about the frequency of the eyes becoming irritated. Clinical examination of the DES was done in line with the procedure undertaken by Schaumberg et al. (2004). Any research aspects that were not covered in the initial questionnaire were modified and included in the questionnaire item. Women who complained of having a constant feeling of dryness or of feeling dry on regular occasions were asked to take a survey based on the newly modified IDEEL dry-eye syndrome complaint module.

A total of 360 postmenopausal women were included for the current study. 177 were postmenopausal women who are currently under HT (estrogen alone and estrogen/progesterone) the other 183 women who were not under HT were considered as a control group. The samples were taken from across 30 gynecological clinics by way of a validated IDEEL questionnaire-based survey study. Most of the study participants were recruited from a database of more than 2,000 postmenopausal women who have been followed during the past 4 years through obstetrics and gynecology clinics from Johns Hopkins, SKMC Cleveland Clinic. The current study identified women who had been menopausal for at least 24 months. These participants had taken at least one of the three most common HT preparations prescribed in the 30 clinics (conjugated



equine E, transdermal E2, or Tibolone) or had taken any estrogen and estrogen/progesterone combination during the same period of time.

The participants in the study (total 360) were subjected to further inclusion criteria as follows. Group 1 incorporated postmenopausal women who had not received HT and had attended the eye clinic without reporting any inflammatory eye condition. Group 2 incorporated postmenopausal women who attended the menopause clinic; it was further grouped into two categories. Group 2A included postmenopausal women who received estrogen HT only for a period of 12, 24, and 36 months. Group 2B consisted of participants who received an estrogen and progesterone combination HT for the same duration as Group 2A throughout the duration of the clinic visits.

The study excluded women with systemic conditions that may cause Parkinson's disease, graft versus host disease, AIDS, thyroid disease, diabetes mellitus, and rheumatoid arthritis. Moreover, patients who had received a bone marrow transplant and those on medications known to cause ocular conditions (e.g., anticholinergic medications, radiation therapy, beta-blockers, and non-steroidal drugs) were also not included for the study. After these exclusions, 360 participants remained who attended the clinic for laboratory blood test and clinical examination. Some of the women who did not take HT decided not to be subjected to this treatment after they attended the menopause clinics. The women were clinically examined and details of their medical history, current medication, alcohol consumption, and family history were captured. An additional exam was performed to record each patient's blood pressure, height, and weight.

In this study, sample was done to ensure random selection from the entire population.

Women who met these criteria were contacted via questionnaires and were later invited to take part in the cross-sectional study. More than 90% of the women who received a questionnaire agreed to take part in the study (a total of 1,800). Eligible sample subjects were postmenopausal women who were above 49 years of age. A continuous cessation of menstrual period for a period of 18-24 months was used as a definitive indicator of menopause in women aged 40 years or older. This criterion was used as inclusion criteria for collecting the data using the cross-sectional questionnaire approach

Sample determination was further made if participants were currently not on any oral contraceptive pills and had not used the pills in the last three months. In addition, subjects were

required not to have used any hormone therapies for at least one week. All subjects needed to report an absence of regular menstrual cycles in the previous 18 to 24 months or to show active signs of perimenopause transition into menopause. Any subject who was pregnant at the time was excluded, as were women using ophthalmic drops within a day of the study visit at the clinics.

The sample subjects enrolled in this study included patients from 30 different clinics including the Tawam Hospital (in affiliation with the Johns Hopkins International Hospital in UAE) and in Sheikh Khalifa Hospital (managed by Cleveland Clinic in the UAE). Confidentiality was ensured for all the subjects by using coded data, and every participant was at liberty to stop taking part in the study at any moment. The study participants also completed a study questionnaire. The questionnaire also assessed history for dry-eye syndrome symptoms and recorded measurements of tear osmolality. The participants completed the questionnaire within duration of two weeks, depending on the provided feedback.

One group used estrogen only HT and another group used a combination of estrogen and progesterone HT. The dependent or measure of outcome is whether the HT use will have reduced dry eye symptoms or reduce severity levels. Even so, a number of cofounders that can increase symptoms of DES were controlled for the current experiment. These include recurrent corneal erosions, various neuropathies, epithelial basement membrane dystrophy, and Parkinson's disease. Other cofounders include grittiness of the eye, tearing, foreign body sensation, and sensitivity to light.

**4.2. Methods:** The use of a survey as the main data gathering method was informed by the understanding that examination of the relation between manifestations in symptoms and signs of DES raises inquiries about the reliability of the various tests. The clinical tests were equally important for establishing the relationship between DES and HT. As a result, the study employed the IDEEL survey questionnaire developed by Abetz et al. (2011). The tool deals with the formulation and authentication of the impact of DES on daily life and evaluates patient-centered outcomes. Thus, the preparation of the tool's measurement was designed to take into account the burden of DES, which is considered to be a contextual necessity in the current study. There is no doubt that the symptoms that are manifested in the OSDI can determine the presence or absence of DES. In addition, a detailed examination of the disease symptoms via the IDEEL survey

ensures that the survey is valid and comprehensive. A patient self-reporting outcome (PRO) measure used to assess the burden of DES on patients to present a cohort assessment of the symptoms of DES was considered as an apt contextual requirement. No doubt, the symptoms presented in Ocular Surface Disease Index (OSDI) assess the presence of DES; however, the detailed list of symptoms in the IDEEL survey ensures the comprehensiveness and validity of the current study.

In addition, the OSDI indexes used to measure the severity of the DES also formed a backbone for understanding the HT relation with dry-eye disease in women. As a new aspect in this area, in each of the 360 study participants, the OSDI was calculated on a scale ranging from 0-100. This validity calculation was performed based on the following OSDI formula:  $OSDI = \frac{\text{the sum of the tallies multiplied by 25}}{\text{all the answered queries}}$ .

Collection of the data was a lengthy procedure given the study-subject requirements. Random sampling was used to identify the various gynecologic clinics under study. Even if the clinical tests achieve to establish a relationship, assessing the severity of the DES is very poor, a study that practically applied the IDEEL survey developed by Abetz et al., (2011).

All data are kept confidential by use of codes, and no personal information was collected. In addition, participants could abandon the study at any moment in time. All information obtained was voluntary, and no participant was forced to give information during the course of the study. Approval was obtained from the Hospitals to conduct research as shown in Appendix A. Informed consent was obtained for the following: a) participation in the survey (attached to the questionnaire in Appendix B); and b) participation in experiment. During sample selection and participant identification, the participants were required to answer the questionnaire post only after they were divided into the relevant groups of estrogen-only-HT users and estrogen/progesterone users.

A questionnaire was used to gather information from the study population about the diversity and potential risk factors of dry-eye symptoms. The reproductive history captured important information about when menstrual periods ended the number of children or pregnancies, history of oophorectomy and hysterectomy. Participants also presented their history of hormone therapy and oral contraceptive use. This information included the name and duration of currently used HT

therapies. No information about duration or type of use was recorded for HT preparations in the past.

63 women identified themselves as current users of HT, although they did not know the type or name of preparation that they were using. This group of women was included in the analysis that involved all of the users of HT, but they were excluded from the analysis of women who were not opposed to the use of estrogen and estrogen/progesterone combinations. The class of natural menopause includes both women that did not cease menstruation, as a result of hysterectomy and those that had a hysterectomy without undergoing oophorectomy.

The questionnaire also collected information about the quantity and frequency of current alcohol use and the history of cigarette smoking. Participants were questioned if they were well informed by their doctors that they had been diagnosed with high blood pressure or diabetes. Diastolic and systolic blood pressures were recorded with a standard sphygmomanometer after the participants had sat for at least five minutes and before any eye drops were administered. The present work defines *hypertension* as a history of diastolic pressure over 95mmHg and/or a history of high blood pressure and/or a systolic pressure that is above 160mmHg.

Detailed information about the use of inhaled or oral steroids was also recorded. The composition of the study participants that did not have the relevant data for the required research variables are as follows: 1% menopausal status; 5% age at menarche; 10% age at menopause; 12% the type of menopause; 4% did not know their history of HT use; 20% the current type of HT preparation; and 5% who were uncertain about the length of time that they had been using the current HT preparation. Additional demographic information was collected to record the ocular history, contact lens use, past surgical and medical history, current ophthalmic and systemic medications, and menstrual-cycle data on the self-reported patient information. Finally, the 7-item questionnaire was also used to collect some diagnostic data. All of the 360 participants reported that they had been diagnosed with an ocular - surface disease or dry-eye syndrome.

The assessment of DES was made using the OSDI and the symptom assessment in the DES questionnaire. OSDI questionnaire include 12 questions on the investigate symptoms of a patient in the last week. The scores range from less severe (0) to more severe (100). Responses were recorded using a visual analogue scale that ranges from 0-100mm. Tear osmolality measurements

were obtained from each eye using the tear-lab osmolality kit (TearLab, San Diego, California). The participants were instructed to cease from rubbing their eyes for 10 minutes. The tear samples were obtained using the Tear-Lab and placing it on the inferior lateral tear meniscus so as to avoid reflex tearing. The osmolality kit was calibrated in accord with the recommendations from the manufacturer. The same investigator made all the measurements to ensure consistency and validity. Because the severity of DES has been reported to be correlated with inter-eye variability, eye measurement that showed high osmolality was incorporated in the analysis of data.

Diagnosis of DES in the current study was made in line with the Copenhagen criteria. When two of the following sets of conditions are met, then the presence of dry-eye syndrome is said to be present. First, Schirmer's test should show abnormal rates of <10mm/5min. Second, the tear-film breakup should be  $\leq 10$ s. Third, the Rose Bengal score should be <4 points. Following informed consent, a brief medical history was obtained and an ocular exam and a brief external examination of the subjects' eyes were also conducted.

A medical specialist was used to perform the Schirmer's test. After 5 minutes of anesthesia using 0.5% proparacaine hydroxide, a Schirmer's strip was positioned at the conjunctival fornix so as to avoid the corneal touch. Though blinking was permitted, the strip was left positioned at this point for a duration of 5 minutes. Using a millimeter scale, the specialist measured the length of the moistened region of the strip. Any reading that recorded less than 5 mm was taken to be a sign of dry eye. In the selected study subjects, the sampling was limited to participants that showed a Schirmer's reading of less than 5mm.

#### **4.2.1- Tear Osmolality measurement**

As noted previously, tear osmolality was measured using the tear-lab osmolality system, which is an *in vitro* device. An *osmometer* is usually a disposable lab kit that holds less than 50 nano-liters of tears for examination (Tomlinson et al. 2006). The generated electrical signals from the laboratory examination are converted into quantitative data that can be displayed as relevant values on a screen. After calibration of equipment at the start of each patient-measurement session, collection of tears was achieved with the help of the same examiner between 8 AM and 11 AM. This collection was achieved through the use of a single-user test card obtained one-on-

one from the eye an hour after removal from the lens and before any drops were applied. There was no use of local anesthesia at this stage. Samples were analyzed directly using the tear-lab osmolality and the recorded values. A total of three recorded measurements were obtained from each eye, and their mean was calculated.

#### **4.2.2 Schirmer's test**

This test was conducted in a confined room with a fan off. No topical anesthesia was applied for this test because it could have altered the results. The eyes were exposed to the test simultaneously using a specialized Whatman's standard filter paper no.41. The filter paper was successively inserted laterally into each conjunctival sac of the lower eyelid using the shortest possible interval of 5 minutes. The paper was read immediately upon removal, and the result was categorized into one of three measures: >10mm-normal eye, 5-10mm suspicious eye condition, and < 5mm-pathological eye.

#### **4.2.3. Tear film break-up time**

A single fluorescein drop of 20g/L was placed into the subjects' eyes. This was attained from a standard minim following the administration of the topical anesthesia. Each patient was required to blink normally multiple times as they placed their head on a slit-lamp support. Afterwards, patients were instructed not to blink as the pre-corneal tear film was evaluated and recorded at approximately  $\leq 10$ -20 magnification. The investigator evaluated the fluorescein that was stained in the pre-corneal tear film while searching for any dry spot in the form of black-gap formation. At the first manifestation of any dry spot, the observer stopped the timer. The mean was then calculated for each separate eye. The findings were interpreted using the Copenhagen criteria: if  $\leq 10$ s, the eye is abnormal; if  $>10$ s, the eye is normal.

#### **4.2.4 Rose Bengal staining**

Following local anesthesia drops, a single drop of Rose Bengal, approximately 10g/L, was placed into both eyes from a standard minim. Subjects were asked to blink normally after placing their head on a slit-lamp support. The staining was examined within 1-2 minutes after the excess pre-corneal film had reduced. Rose Bengal staining was graded using the Bijterveld method. The ocular surface is categorized into 3 areas: cornea, temporal bulbar and nasal bulbar conjunctiva. This system grades the stains on the exposed sections of the cornea from 0 to 3: where if the value is 0 the result indicates that there was no staining, 1-slight staining, 2-moderate staining,

and 3-intense staining. Adding up these scores gives ranks were maximum of 9 on all the three stained sites of the cornea.

#### **4.2.5 Statistical Analysis**

Analysis of the data was achieved by using SPSS version 21.0 software.. When the samples were small, Fisher's Exact was appropriate. Expression of the data was performed through the use of percentages and numbers (categorical) or by the standard deviation and the mean. Biochemical and demographic features of population under study and occurrence of the dry-eye syndrome, according to HT status were compared using a 2-sample t-test. The Pearson's chi-squared *test* ( $\chi^2$ ) test was used to measure the categorical variable. The prevalence of glaucoma, cataract or dry-eye syndrome was estimated for all study subjects. Where there was diabetic retinopathy, the data was examined only for those subjects with diabetes. The variation in demographics was analyzed using t-test for continuous measures. The variations in the measures of outcomes between postmenopausal women were examined via the paired t-tests. Bias-corrected percentiles and boot strapping with 2000 repetitions were used to generate the 95% confidence interval.

In the case of the tear osmolality measurement the difference was assessed using the one-way analysis of variance (ANOVA). Further, recently utilized cut-off of 308mOsm/L (Lemp et al., 2011), variation in low versus high tear osmolality between the groups was examined via Fisher's exact test. Since the range of severity levels was based on percentile deviation, the ocular-surface disease index scores for postmenopausal women in Groups 2A and B and their corresponding Group 1 were assorted under severe levels (>75), moderate (falling in and around 50) and mild (<25). These results were further subjected to statistical analysis using SPSS software. Post hoc analysis, ANOVA, and descriptive statistics using Welch and Brown-Forsythe tests were performed.

## 5. Results

### 5.1. Participant Demographics

360 postmenopausal women (aged 49 years and above) were enrolled into the study. The majority of the women surveyed (55%) was recruited from Johns Hopkins hospital and were aged between 50 years and 64 years. The rest of the study participants were surveyed at Cleveland clinic. Most women (51.1%) had attained graduate degrees and had taken more than a single eye test examination at a clinic. The distribution of age, education, and eye test frequency are summarized in Table 1.

**Table 1: Relationship between demographic and social characteristics and HT among postmenopausal women**

Characteristic	Number and Percentage %	
	Total	HT Users
Age, years		
≤49	11 (3.1)	7(63.6)
50 – 54	104 (28.9)	60 (57.7)
55 – 59	119 (33.1)	58 (48.7)
60 – 64	97 (26.9)	41 (42.3)
> 64	29 (8.1)	11 (37.9)
Education		
Primary	25 (6.9)	10 (40)
Intermediate	22 (6.1)	9 (40.9)
Secondary	32 (8.9)	14 (43.8)
Graduate	223 (61.9)	114 (51.1)
Post Grad	58 (16.1)	30 (51.7)
Income		
<10000	7 (2)	1 (13)
10000 – 30000	36 (10)	9 (24)
30001 – 60000	94 (26)	43 (46)
60001 – 90000	166 (46)	90 (54)
>90001	58 (16)	34 (60)
Eye test Frequency		
≥1 in 2 years	306 (85)	156 (51)
<1 in 2 years	54 (15)	21 (39)



All of the study participants were accounted for based on follow-up data obtained for both the study and control groups throughout the study period. 260 women Group 1 (control group) was composed of 183 participants who did not receive HT therapy and did not report any inflammatory eye conditions or irritations. Group 2A consisted of 92 women who received estrogen-alone HT for duration of 28 months. Group 2B consisted of 85 postmenopausal women that got treated with estrogen and progesterone HT for a treatment period of 36 months and were examined at intervals of 12, 24, and 36 months. In addition, the use of HT is higher in high-income women and in those with higher academic qualifications. This also means that the frequency of eye exams in postmenopausal women under the use of HT is higher than it is in women who are not using HT in the last 36 months irrespective of the dosage and duration of HT. The normal range for women with dry eye was only about 8.9% of the OSDI index that is below the score of 11.5, based on the OSDI index in establishing the presence or severe, moderate, and mild symptoms of the DES among the other participants.

The variations in postmenopausal treatments and clinical test results are also presented in Table 2.

**Table 2: Age distribution and the duration of menopause in the groups**

Groups	Age		Duration of menopause	
	M±SD	<i>p</i>	M±SD	<i>P</i>
<b>Study group 2A HT(+) Estrogen Only</b>				
1. Dry eye(+)	54.2 ± 1.8	>0.05	3.1 ± 01	0.01
2. Dry eye(-)	55.7 ± 1.9		2.3 ± 07	
<b>Study group 2B HT(+) Estrogen/Progesterone</b>				
1. Dry eye(+)	55.4 ± 1.8	>0.05	5.2 ± 09	0.01
2. Dry eye(-)	55.3 ± 1.9		2.4 ± 03	
<b>Control group 1 HT(-)</b>				
3. Dry eye(+)	54.0 ± 1.5	>0.05	3.1 ± 06	0.014
4. Dry eye (-)	55.1 ± 1.8		2.7 ± 04	

Table 2 shows that the mean year for Group 1 (Control group) is 54.0 ± 1.5 years for those with dry-eye disease and 55.4 ± 1.8 years for women without dry-eye disease. The women with dry-eye syndrome in this group have a minimum duration of 3 years post-menopause; those without

dry-eye disease have a minimum duration of 2.7 years post-menopause. Group 2A consisted of postmenopausal women with a mean age of  $54.2 \pm 1.8$  years with a post-menopause duration of 3 years. In Group 2B, postmenopausal women with dry-eye syndrome had a mean age of  $55.4 \pm 1.8$  years with a post-menopause duration of 5 years. Table 3 shows the ANOVA test results on the variety of the postmenopausal treatments.

**Table 3: ANOVA test results examining the variance in postmenopausal treatments**

Difference in postmenopausal treatments	N	M±SD	Standard Error	95% CI for mean		p
				Lower value	Upper value	
Non-HT	183	22.76±11.92	0.881	21.0182	24.4976	< 0.001*
HT Treated with estrogen only (O)	90	92.52±12.93	1.363	89.8059	95.2252	
HT Treated with estrogen and Progesterone (O and P);	87	50.89±12.44	1.334	48.2394	53.5445	

\* Values are expressed at 95% confidence interval and are based on separate ANOVA and Welch and Brown-Forsythe tests for each outcome [menopausal treatments between non-HT, HT estrogen only (O) and HT estrogen and Progesterone (O and P)]

In every clinic, a single clinical examiner performed the DES diagnoses on the subjects. 175 (48.6%) participants reported suffering from DES signs like burning, irritation, and itching in their disease history. Findings (from the self-report group) indicated 50.8% healthy participants that did not present with any ocular inflammatory conditions and a 49.2% population that reported with dry-eye symptoms. A total of 67% had not taken any HT medications (such as tibolone or transdermal E2, and conjugated equine E) in the 4 months prior to the study. The same population had not used any medication such as antidepressants, antihistamines, and inhaled or nasal steroids in the last 30 months. Table 4 shows the ANOVA test results that examine the dosage levels of HT administration in the study subjects.

Table 4 shows a statistically significant relationship ( $p < 0.001$ ) between subjects who took  $< 1\text{mg/day}$  of HT and those who were treated with  $> 1\text{mg/day}$ .

**Table 4: ANOVA test results examining dosage levels of HT administration for postmenopausal women.**

Difference in dosage levels of HT administration	N	M±SD	Standard Error	95% Confidence Interval of Mean		P-Value
				Lower Bound	Upper Bound	
<1mg/day	71	69.03±25.48	3.02	63.0008	75.064	* < 0.001
>1mg/day	106	74.08±23.56	2.281	69.5432	78.6209	

\* Values are expressed at 95% confidence interval and are based on separate ANOVA and Welch and Brown-Forsythe tests for each outcome

Dosage levels <1 mg/day and > 1mg/day between HT O and HT Estrogen and Progesterone  
 The ANOVA test results for the 36 months treatment duration are shown in Table 5. The administration of HT throughout the treatment duration shows statistical significance (p< 0.001) in the administered HT on the study group.

**Table 5: ANOVA test results examining the variance in the duration of HT administration for postmenopausal women**

Difference in duration of HT administration	N	M±SD	Standard Error	95% CI		P-Value
				Lower limit	Upper limit	
12 Months	47	61.23±25.93	3.78	53.61	68.84	< 0.001
24 Months	44	77.64±24.24	3.65	70.27	85.01	
36 Months	48	75.12±21.95	2.36	70.41	79.82	

\* Values are expressed at 95% confidence interval and are based on separate ANOVA and Welch and Brown-Forsythe tests for each.

Dosage levels <1 mg/day and > 1mg/day between HT O and HT O and P; and duration 12, 24 and 36 months between HT O and HT O and P.

As shown in Table 6, in study groups A and B, a total of 175 (48.9%) participants presented with dry-eye symptoms. Group A was comprised of 52.6% of the subjects, while Group B was comprised of 48.4% of the subjects. In the control group, 52.1% of the control subjects did not undergo hormone therapy during the treatment period of 36 months. Table 6 presents the clinical findings in these groups after the first 12 months of therapy treatment.

**Table 6: The dispersal of the DES conditions in the study and control groups after 12 months of HT use**

Groups	Initial exam		12 month exam		<i>p</i>
	No. of cases	Dry eye	No. of cases	Dry eye	
Study 2A HT(+) Estrogen	90	90	90	90	0.003
	90	0	90	85	
Study 2B HT(+) Estrogen/progesterone	87	87	87	83	0.003
	87	0	87	77	
Control HT(-)	183	183	183	0	1.0
	183	0	183	0	

Table 6 shows the dry-eye findings after a medication period of 12 months. The results showed no significant difference in Meibomian dysfunction ( $p>0.25$ ) in both test groups.

**Table 7: Clinical results, DES findings, and the levels of serum hormone 1 after 12 months**

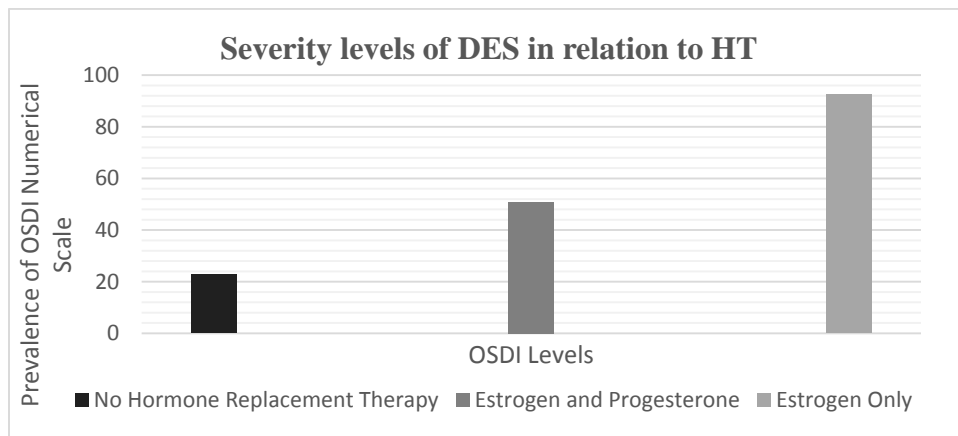
	Study (patients on HT)				Control		<i>p</i> -value
	Group 2A (with dry eye), <i>n</i> = 90		Group 2B (with DES), <i>n</i> = 87		Group 1 (without DES), <i>n</i> = 183		
	Before HT	12 months after HT	Before HT	12 months after HT	Before research h	After 12 months	
Meibomian dysfunction (grade)	2.1 ± 0.3	2.2 ± 0.4	1.4 ± 0.1	1.8 ± 0.3	2.4 ± 0.1	2.3 ± 0.2	0.42
Schirmer's with anesthesia (mm)	3.8 ± 1.2	3.1 ± 1.0	4.4 ± 1.4	4.9 ± 1.2	11.3 ± 0.7	11.2 ± 0.8	0.73
Bengal staining (BS)	4.0 ± 0.1	4.2 ± 0.1	3.6 ± 0.3	4.2 ± 0.2	3.7 ± 0.2	3.9 ± 0.3	0.68
Estradiol (pg/ml)	32.7 ± 7.3	56.8 ± 11.2	35.5 ± 6.3	63.3 ± 10.7	37.3 ± 3.8	37.5 ± 3.3	0.49
Tear film break-time (BUT) (s)	4.2 ± 0.8	4.1 ± 0.4	7.3 ± 2.2	7.8 ± 1.2	14.1 ± 0.8	14.3 ± 0.8	0.45
LH (μ/ml)	39.5 ± 4.2	37.3 ± 4.2	37.1 ± 2.0	31.3 ± 2.1	39.4 ± 3.2	33.3 ± 2.8	0.73

Table 7 shows that the number of subjects with Schirmer's >5mm was the similar in Group 2A (26%) and Group 2B (25%). Therefore, no statistically significant difference was found after using HT ( $p<0.73$ ). Besides, estrogen showed no correlation with tear osmolality ( $p<0.51$ ,

p<0.08) or with the subjects' symptom scores (BUT p<0.77, p<0.05; SANDE frequency p<0.04, p<0.83; SANDE severity p<0.07, p<0.50). In addition, the tear osmolality and OSDI were not correlated (p<0.04, p<0.81).

## 5.2 Severity Levels of DES in Relation to HT

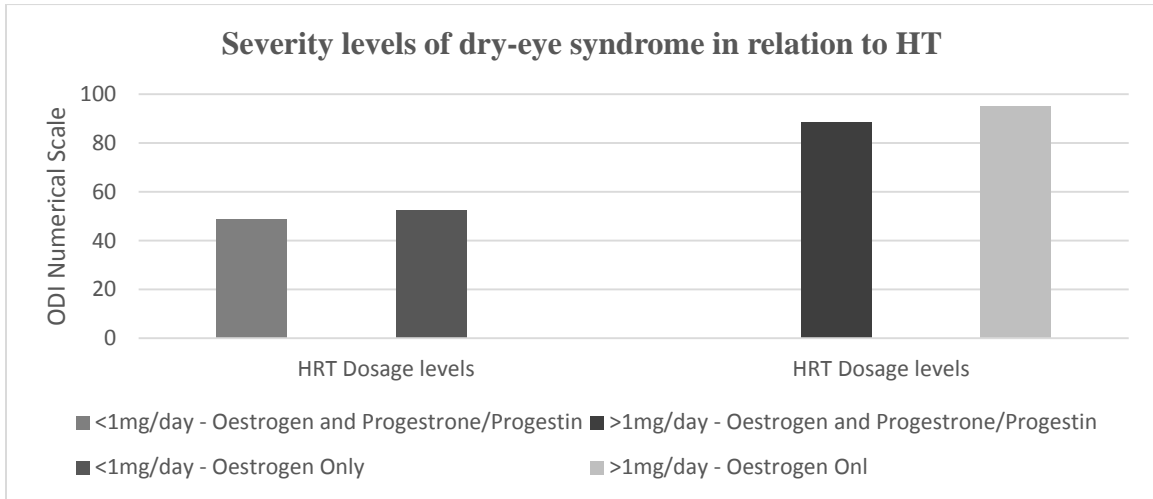
The severity levels of DES and their relationship to HT treatment are shown in Figure 1. Women who were administered estrogen-only HT appear to have a higher OSDI than subjects who used a HT combination of estrogen and progesterone.



**Figure 1: Severity levels of dry-eye syndrome (DES) in relation to Hormone therapy (HT) usage and non-usage among 360 Postmenopausal women.**

Users of HT include only current users with the most recent assigned therapy. Variation in the severity levels of DES across the three categories of HT were significant in accordance with the severity definition of mild, moderate, and severe (p<0.001).

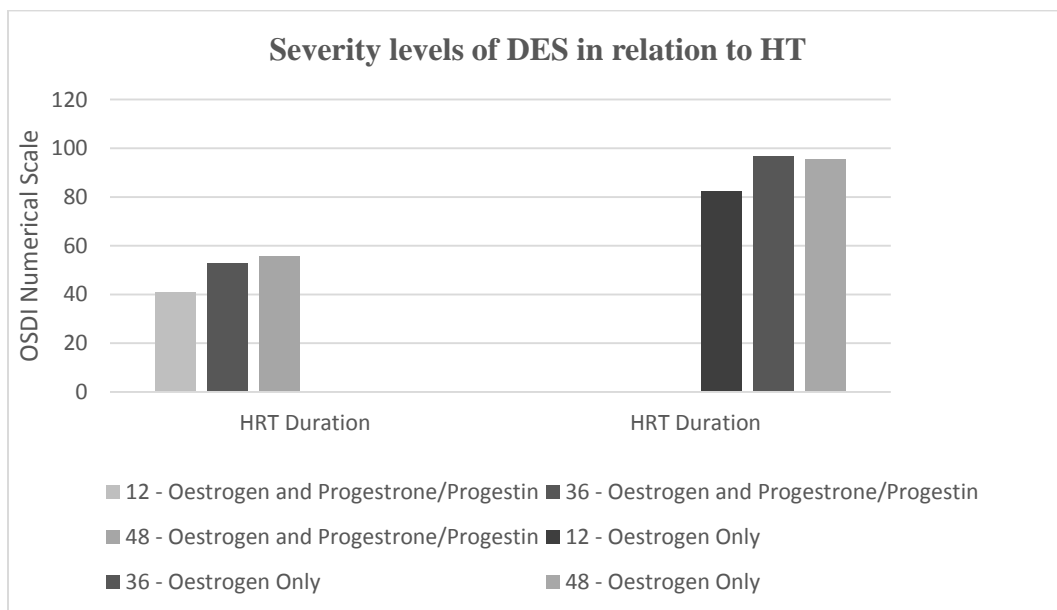
Figure 2 shows the severity levels of DES in relation to HT medication. The severity levels appear to be higher in subjects who used more than 1 mg/day of estrogen-only HT than in subjects who took the same dosage of estrogen-and-progesterone HT. In addition, the severity is higher in subjects who took less than 1 mg/day estrogen-only HT than in those who took the same dosage of estrogen and progesterone.



**Figure 2: Severity levels of dry-eye syndrome in relation to hormone therapy (HT) dosage levels among 360 postmenopausal women.**

Dosage levels include only current users with the most recent dosage levels. Variation in the severity levels of DES across the two categories of HT were statistically significant ( $p < 0.001$ ) in accordance with the dosage definition of  $< 1$  mg/day and  $> 1$  mg/day.

Figure 3 shows DES severity levels among the 360 postmenopausal women. Women who received estrogen-only HT in this duration appear to show higher severity levels than other study subjects. Prolonged use of HT appears to increase severity levels of DES in postmenopausal women.



**Figure 3: Severity levels of dry-eye syndrome in relation to hormone therapy (HT) duration among 360 postmenopausal women.**

### 5.3. Observed eye changes to hormone therapy use

Among the study group subjects, and for the three measurements taken (Schirmer test, Tear Breakup Time (BUT), and Rose Bengal), the mean values for DES were slightly worse after 12 months of HT treatment. See Table 7. Patients on a combination of estrogen/progesterone showed more improvement than patients on estrogen alone after 12 months of HT use, although the discrepancy was not statistically significant. The results are summarized in table 8 after 36 months of HT use.

**Table 8: Differences in dry eye assessments among subjects after 36 months of HT use**

	Study groups	Control group	Difference Mean $\pm$ SD	<i>p</i> -value
Tear osmolality (mOsm/L)	5.2 $\pm$ 25.1	3.0 $\pm$ 7.4	2.2 $\pm$ 5.7	0.43
OSDI* score	1.5 $\pm$ 6.2	1.1 $\pm$ 0.4	0.4 $\pm$ 5.8	0.74
SANDE* severity score	2.1 $\pm$ 10.1	1.4 $\pm$ 1.3	0.7 $\pm$ 8.8	0.69
SANDE frequency score	3.1 $\pm$ 7.1	1.2 $\pm$ 8.7	1.4 $\pm$ 8.8	0.48

**OSDI\*- Ocular-surface disease Index**

**SANDE\*- Symptom Assessment in Dry Eye**

The results of the severity measurements after 36 months of HT use are presented in Table 8. The OSDI score in the study group was (<57) with a mean of 1.5 indicating a moderate state of the dry-eye disease. However, after 36 months of HT use, there was no significant difference in OSDI and tear osmolality ( $p > 0.27$  for both). Likewise, the proportion of the study participants with tear osmolality  $> 307$  mOsm/L was found to be similar both in test groups that used estrogen HT alone and in those that used a combination of estrogen/progesterone HT use ( $p < 0.77$ ).

**Table 9: The comparative assessment of the dry eye by HT use in 12, 24, and 36 months**

	N	Tear Osmolarity (mOsm/L)	Ocular-surface disease Index	Symptom Assessment in Dry Eye	
		Mean± SD	Median (25%, 75%)	Severity Median (25%, 75%)	Frequency Median (25%, 75%)
All (n)	177	302.5 ± 14.3	4.6 (0.1, 10.3)	7.1 (1.1,19.1)	12.1 (4.0, 26.0)
HT use (12 months)					
Group 2A	90	302.7 ± 14.3	5.4 (1.2, 14.7)	11.0 (2.0,28.0)	17.5 (7.0, 29.0)
Group 2B	87	301.2 ± 13.7	4.3 (1.0, 7.6)	11.0 (1.0,12.0)	17.1 (1.0, 22.0)
Group 1	183	300.1 ± 12.6	4.2 (1.0, 7.3)	2.0 (0.0, 12.0)	6.3 (1.0, 23.0)
Difference 95% CI*		2.7 [-3.0, 8.3]	1.2 [-2.2, 5.3]	8.0 [2.3, 18]	12.5 [2.3, 19]
p-value*		0.39	0.27	0.02	0.009
HT use (24 months)					
Group 2A	90	303.9 ± 16.5	4.7 (1.0, 13.7)	12.7 (6.3,32.3)	23.0 (9.3, 32.0)
Group 2B	87	302.7 ± 12.5	4.6 (1.1, 12.2)	2.1 (0.1, 11.2)	6.1 (1.1, 14.0)
Group 1	183	299.7 ± 12.3	4.6 (0.0, 8.2)		
Difference 95% CI*		4.3 [-1.7, 9.7]	0.04 [-2.1, 4.2]	10.5 [6, 17]	15.1 [9, 21]
p-value*		0.16	0.54	0.0001	<0.0002
HT use (36 months)					
Group 2A	90	305.4 ± 17.5	5.3 (2.1, 14.6)	14.0 (7.0,33.0)	22.5 (10.0,40.0)
Group 2B	87	302.7 ± 16.3	4.7 (0.0, 13.7)	12.1 (0.0,12.0)	20.3 (10.1,35.0)
Group 1	183	299.9 ± 11.7	3.3 (1.0, 10.6)	6.0 (1.0,11.0)	8.0 (1.1, 21.0)
Difference 95% CI		5.7 [-0.02, 11.6]	1.2[-1.3,7.2]	12.1 [7, 29]	25.5 [6.5, 26.1]
p-value		0.053	0.37	0.0003	0.0003

The number of estrogen and estrogen/progesterone therapies administered ranged from <1 mg/day to >1 mg/day. At 24 months of HT use, in both cases, the estrogen HT alone and estrogen/progesterone HT doses were found not to correlate with tear osmolality ( $p < 0.48$ ;  $p < 0.12$ ). In addition, results indicate that HT doses do not correlate with symptom scores (SANDE frequency  $\rho = -0.04$ ,  $\rho = 0.81$ ; SANDE severity  $\rho = 0.06$ ,  $p = 0.52$ ; OSDI  $\rho = 0.54$ ,  $p = 0.79$ ). Table 5 shows the results for HT use after 36 months.

As shown in Table 9, *post hoc* analytical comparisons at 12, 24, and 36 months indicate that the mean score from the postmenopausal women who were not under HT (control group) is as follows ( $M = 22.73$ ,  $SD = 11.93678$ ). This result is significantly different from that obtained from the postmenopausal women under estrogen HT only ( $M = 91.53$ ,  $SD = 12.82622$ ) and from that obtained from the postmenopausal women that used a combination of estrogen/progesterone HT ( $M = 50.78$ ,  $SD = 12.33477$ ). Moreover, Table 9 shows that the *post hoc* correlation indicates that



the postmenopausal women who used <1mg/day HT dosage had a mean score of (M=61.22, SD = 25.37121). These results are significantly different compared to the findings from postmenopausal women that used >1mg/day HT dosage (M = 74.171, SD = 23.45657). *Post hoc* comparisons also show that the mean score for postmenopausal women after 12 months HT treatment (M = 61.12, SD = 25.94528) was significantly different than that for postmenopausal women after 24 months HT treatment (M = 77.532, SD = 24.13282) and 36 months HT treatment (M = 75.225; SD = 21.84143).

After 36 months of HT use, comparison of the ocular surface and OSDI questionnaire was analyzed. The results (Tab.10) were analyzed as a median range using the Dunn Post-test and a Kruskal-Wallis test for each of the variable. The ocular-surface disease index is indicated in milliliters (mm), and the break-up time (BUT) given in seconds (s). The bold p-values show a statistically significant correlation. As shown in Table 10, after 36 months of HT use. The OSDI questionnaire indicates a statistically significant correlation (p< 0.006). This shows a correlation between the dry-eye syndrome in the control group and the study groups that used HT. However, there was no statistically significant association between HT use and tear osmolality (p<0. 304) and Schirmer’s results (p<0. 378). In comparison, there is a statistical relation (p<0. 0001) between HT use and tear breakup time. The results were obtained after the 36 month study period by asking the study subjects to give self-reports on their DES situation before and after the use of HT.

Table 10: Comparison of the ocular surface evaluation and OSDI questionnaire in study and control groups

	Control group	Estrogen HT Only	Estrogen and Progesterone HT	p-value
OSDI questionnaire	3.1 [0.0–34.0]	8.6 [0.0–24.0]	10.4 [0.0–38.0]*	<b>0.006</b>
Tear osmolality (mOsm/L)	303.4 [274.0–332.0]	293.0 [276.0–343.0]*	297.4 [283.0–325.0]*	0.304
Schirmer’s test (mm)	11.0 [1.0–2.0]	6.1 [1.0–2.0]	8.0 [1.0–1.0]	0.378
Tear BUT (s)	13.0 [7.0–9.0]	7.0 [5.0–14.0]*	8.3 [4.0–11.0]*	<b>&lt;0.0001</b>
Rose Bengal staining	1.0 [1.0–1.0]	1.0 [2.0–3.0]*,§	2.0 [1.0–2.0]*,§	<b>&lt;0.0001</b>
Fluorescein staining	1.0 [0.0–2.0]	1.0 [1.0–4.0]*,§	3.0 [1.0–4.0]*,§	<b>&lt;0.0001</b>

Symptom Assessment in Dry Eye				
SANDE Frequency	21.5 [2.0–82.0]	18.5 [4.0–77.0]	21.4 [5.0–87.0]*	<0.037
SANDE Severity	9.1. [4.0–5.0]	8.9 [4.6.0–4.7]§	10.0 [1.20–2.10]*.§	<0.000 1

1. \*  $p < 0.05$  in comparison with the control group.
2. §  $p < 0.05$  between estrogen HT only and estrogen subjects.

During symptom assessment for dry eye (SANDE), participants reported reduced frequency ( $p < 0.037$ ) and severity ( $p < 0.0001$ ) of dry eye after using HT. However, there was a negative correlation in patients who used estrogen HT alone and reported insignificant changes throughout the 36 months ( $p < 0.378$ ) compared to the estrogen / progesterone group that reported significant changes after using HT combination. Upon taking the study questionnaire, 38% (72 subjects) of the control group that did not take any HT medication during the study demonstrated some dry-eye symptoms.

#### 5.4. Questionnaire results of the patients before and after HT

Table 11: Ophthalmic questionnaire results pre- and post-HT

Score	Before HT			After HT		
	Median $\pm$ SEM	Min	Max	Median $\pm$ SEM	Min	Max
Cornea (0–3)	1 $\pm$ 0.2	0.0	2.9	1 $\pm$ 0.1	0	3
Tearing (1–4)	0.5 $\pm$ 0.2	0.0	2.0	0 $\pm$ 0.1	0	2
Mucoid secretion (1–4)	1 $\pm$ 0.2	1.1	3.0	1 $\pm$ 0.2	1	1
Foreign-body sensation (1–4)	1 $\pm$ 0.2	1.0	2.0	1 $\pm$ 0.1	1	1
Conjunctiva (0–4)	1.4 $\pm$ 0.2	1.0	2.0	1 $\pm$ 0.2	1	2
Burning (1–4)	2 $\pm$ 0.2	1.0	3.0	2 $\pm$ 0.1	1	2
Redness (1–4)	1 $\pm$ 0.2	1.0	2.0	1 $\pm$ 0.2	1	3

A physical exam and final questionnaire analysis of the mucoid secretion, tearing, burning, redness, foreign-body sensation, cornea, and conjunctiva revealed a number of findings shown in Table 11. These findings show that women who take HT have less DES complaints than women that do not receive any HT or sex-hormone-related therapies. The questionnaire test after the 36 months of medication shows more DES complaints from the control group. It registered 38% new DES complaints that were initially absent at the start of this study.

## 6. Discussion

Sexual hormone changes have shown to exacerbate the risk of DES (Avasthi and Luthra, 1967; Affinito et al., 2003; Chia et al., 2013). However, an association between tear production and hormone changes in postmenopausal women is obviously complex (Abetz et al., 2011). Years of research have not revealed how changes in sex hormones during menopause come together to cause ocular changes (Zafar et al., 2010; Tseng and Tsubota, 2011). According to Warren (2009) this relationship has to do with direct changes on the ocular region, meibomian gland and lachrymal gland (Warren, 2009). Therefore, lack of hormones, or their reduced production, may contribute to DES.

In postmenopausal women with dry-eye symptoms, the use of HT (estrogen alone, or estrogen and progesterone) has been cited as an appropriate way to ease DES symptoms (Abelson et al., 2000; Altintas et al., 2004). Nonetheless, this claim has never been significantly proven because of conflicting reports on the efficacy of hormone therapy on DES. The objective of the current study was to perform a cross-sectional comparative study to study the relationship that exists between HT and DES in women that have attained menopause. This was achieved by comparing the occurrence of DES in participants that were treated with HT intervention and those not treated with the same intervention for a period of 3 years. This cross-sectional study used the OSDI analytical method and an IDEEL survey to explore the link between DES and HT in postmenopausal patients for a treatment period of 36 months. Tear-film breakup time, Schirmer's test, and Rose Bengal staining were performed on all the study subjects. Findings from the study indicate that DES is less common in patients who take estrogen HT only (Tab... ), though the results were not statistically significant from the control group ( $p < 0.0$  ). However, self-reports of dry eye after 36 months were found less commonly in participants that used estrogen and progesterone HT. These findings negate the assumption that use of HT (especially estrogen alone) is a protective health management approach against DES in postmenopausal women.

These findings add a unique contribution to the literature on HT use and DES in postmenopausal women, considering the dosage and duration of HT treatment. On duration, women who take estrogen HT alone (<1 mg/day) indicate an improved outcome after 12 months; but DES symptoms appear to decline with prolonged use of HT. In contrast, women who take a

combination of HT (estrogen/progesterone) experience improved DES with time when using (>1 mg/day).

However, postmenopausal women not using HT are at a higher risk of DES; 38% of the study subjects reported new cases of DES symptoms at the end of the study duration. Another studies (Schaumberg, et al., 2007; Schaumberg, et al., 2003; Becquet, et al., 1997; Stahl, et al., 2009) based on the severity of DES also showed a significant association between DES and HT. However, most of the women in the present study showed mild to severe DES symptoms and therefore is in a consistent with the findings from previous studies (Schaumberg, et al., 2007; Schaumberg, et al., 2003; Becquet, et al., 1997; Stahl, et al., 2009) which have empirically validated the relationship between DES and HT.

### **6.1. Mean Schirmer's test**

Following the initial 12-month study period, the Schirmer's score of the study groups for both the left and right eyes (n=358) has less significant changes ( $p < 0.73$ ). (Table 7). However, self-reported questionnaire analysis indicated reduced DES symptoms, as shown by a slight improvement in Schirmer's test at 36 months (Table 8), although the change was statistically insignificant ( $p < 0.378$ ). Tear function decline was observed mostly in the estrogen-alone HT group (Group 2A). This observation means that the use of HT reduces tear production; the impact is more on postmenopausal women that use estrogen-only therapy. These findings are similar to those of Versura and Campos (2005), who noticed increased deterioration of DES among women who use estrogen therapy for a prolonged period.

Based on this observation, suitable advice is that postmenopausal women who are considering or taking HT should be made aware of the increased risk of DES with estrogen-only therapy. According to Toker et al. (2003) estrogen-only HT works to decrease intraocular pressure. Interestingly, estrogen and progesterone HT combination has no effect on intraocular pressure. In the current study, using a combination of estrogen and progesterone therapy showed a fewer DES symptoms after 36 months of treatment registered than those under estrogen-alone (Toker et al., 2003).

In both groups (Group 2A and Group 2B) the mean value of Schirmer's test failed to indicate any statistically significant changes after 36 months of intervention (Table 7). Before therapy, the Schirmer's value for the left and right eye for Group 2A (estrogen-only) was 8.8 mm and 8.3 mm, respectively. In contrast, the Schirmer's value of the right and left eye for Group 2B (estrogen and progesterone) was 9.4 mm and 9.9 mm, respectively. After therapy, the Schirmer's value was 9.7 mm and 10.1 mm from the left and right eyes in Group A, and 11.3 mm and 11.6 mm from the right and left eyes in Group B, respectively.

Statistical analysis indicates that women on estrogen-only therapy experienced an insignificant change throughout the 36 month medication period. This indicates that women under estrogen therapy are at a significant risk compared to postmenopausal women with estrogen and progesterone who had a significant mean change in the Schirmer's value ( $p < 0.001$ ). See Table 10. Minor variation in Schirmer's value after 36 months of medication duration clearly shows that women under HT treatment need to reassess their acceptance of the postmenopausal hormone-based therapies, so as to avoid unnecessary ocular surface discomforts.

This approach is consistent with studies that have shown a possible relation between prolonged HT use and the risk of DES for women under estrogen-only therapy (Zafar et al., 2010; Barney, 2002). Furthermore, the insignificant change in Schirmer's test after 3 years of HT treatment largely refutes the study findings that have failed to establish a correlation between HT and DES (Burton et al., 2004; Chalmers and Begley, 2006). Individually, there was also great variation in the Schirmer's test between both study groups ( $n=175$ ) in comparison to the control group ( $n=183$ ). As the mean value in the right and left eyes of the study group was 8.1 mm and 8.4 mm before HT therapy; after the therapy it was 9.3 mm and 9.5 mm for the left and right eyes, respectively. In comparison, the mean Schirmer's test in the controls at the start of the research was 12.7mm and 13.3mm for both left and right eyes, respectively. After the 40 month study duration, the average Schirmer's value for the control group was 12.8mm and 13.2mm for the left and right eyes.

The contrast existing in the study between patients who treated with HT and the controls showed that the mean values of the Schirmer's test failed to elaborate potential changes ( $p < 0.352$ ). In addition, the Schirmer's values was not improved between Group 2A and Group 2B ( ). Also, the test used to examine the quality and quantity of tears fails to establish a statistically

significant response to HT therapy between the baseline and final Schirmer's values. Besides, the tear-break-up-time value was within the normal range before and after the 36-month treatment period. Therefore, this study demonstrate that HT does not significantly reduce DES symptoms in postmenopausal women and these finding are in accordance with various past study (Burton et al., 2004; Chalmers and Begley, 2006).

## **6.2. Tear Breakup Time**

The current study found an insignificant effect of HT on tear break-up time ( $p < 0.05$ ) at the 12 month assessment period (Table 3). There was a weak relationship between tear break-up time in the women under estrogen-only HT at 36 month treatment duration ( $r = -0.231$ ,  $P > 0.05$ ). This negative correlation is also found in the group under estrogen/androgen HT therapy ( $r = -0.312$ ,  $P > 0.05$ ). Similarly, there was a weak positive association between the control group ( $r = 0.261$ ,  $p > 0.05$ ) and the study group ( $r = .263$ ,  $P > 0.05$ ) after the treatment period. Nevertheless, no significant relationship was obvious in the tear break-up time between the estrogen-only group and the estrogen and progesterone HT group ( $P > 0.05$ ). These findings indicate that prolonged use of HT therapy does not significantly improve the DES condition.

Before the therapy, the tear break-up time was 4.2 seconds. It was 7.0 seconds after the therapy in the estrogen-only participants compared to the estrogen-and-progesterone group as the tear break-up time was 7.3 seconds before and 8.3 seconds after the therapy. In contrast, at the 36 month medication period, the tear break-up time was 4.1 seconds for estrogen-only and 7.8 seconds and estrogen/progesterone HT groups. This finding shows a statistically significant change ( $p < 0.0001$ ) from the control group after HT use (Table 6). Others (Taner et al., 2004) shows a significant effect of HT (estrogen and progesterone) on DES, since the mean tear break-up time for the estrogen-only group fell in the normal range both before and after HT therapy.

However, the estrogen-alone HT approach appears to have a weak negative correlation with the tear break-up time, showing a potential risk of escalating DES symptoms in postmenopausal women. According to Worzala et al. (2001), the Birmingham study failed to establish improvement in tear quality among research subjects. Subsequently, a Kupperman index undertaken by Vavilis et al. (2007) on the 11-candidate with menopause symptoms both before

and after 12 months of HT use showed significant DES symptom relief by a combination of Tibolone and conventional HT, as opposed to estrogen use as a single HT.

Vavilis et al., (2007) came to the same result and confirmed the efficacy of a combined HT use over estrogen-only therapy in postmenopausal women with DES

HT improve, insignificantly, eye symptoms according to a self-report questionnaire analysis (Tab.7). For example, eye burning, and redness of the eye, was effectively treated in the estrogen and estrogen/progesterone groups, while the control group reported 72 new cases of irritation after 36 months. This irritation can be attributed to increased IgA levels during the study duration. According to Kuscu et al. (2003) the use of HT can significantly increase the tear IgA levels over a prolonged treatment period. Therefore, the therapies have no detrimental effect on the eye symptoms, according to the results from the ophthalmic questionnaire (Table 7).

These results are consistent with the findings by Tomlinson et al. (2001), who studied the impact of HT on tear physiology changes result from IgA and tear lysozyme in postmenopausal women that are under HT treatment. Similarly, Taner et al. (2004) failed to establish any correlation between HT and tear break-up time. Nor did they identify any association between HT and Schirmer's test values in postmenopausal women. Nonetheless, Altintas et al. (2004) reported an increase in tear quantity and quality in women who were under two years of HT. These observations conflict with current study findings that ran for the same duration (n=358). To confirm the current observations additional medication, duration and research subjects are required.

### **6.3. Tear Osmolality**

Currently, the consensus in the literature is that the normal osmolality value of a non-DES individuals is approximately  $302 \pm 9.7$  mOsm/kg. This value can differ from 283 to 318 mOsm/kg based on different measurements. Kuscu et al. (2003) report large variations in the values of osmolality among DES individuals with a mean of  $325 \pm 21.2$  mOsm/kg ranging from 315 to 366 mOsm/kg. The meta-analysis study indicates a cutoff value of 315.7 mOsm/kg, which is similar to subjects that used estrogen-only therapy versus 315.2 mOsm/kg in estrogen/progesterone group over the 36 month treatment period (Kuscu et al., 2003). The results

in the present study (Table. 4 and 5) showed that tear osmolality is consistent with the study conducted by Kuscu et al., (2003), whereas, there are a slightly higher than the results (308.3 mOsm/kg) reported by Tomlinson et al. (2001). The high value of this study may result from the tear production and reduction in volume in postmenopausal women with an age greater than 45 years compared to the mean age of <42 years used by Tomlinson et al. (2001). As a result, this high volume may have increased the solutes in the tears and resulted in an increase in tear osmolality. Given the mean age of more than 40 years in the current study, the mean osmolality could also be slightly higher than the normal values of tear osmolality. Despite this, current reference values for the different age groups are non-existent (Chia et al., 2013). This lack warrants further research on the topic.

In addition, there was no significant OSDI correlation in study Group 2A, which indicates that moderate to mild forms of DES exhibit different grades (Srinivasan et al., 2007). Nonetheless, the current study was able to elaborate the overall difference between the control and the study groups. It is important to ensure that there is a constant humidity and room temperature during the process of drying the tears, as the process of tear osmolality measurement can be changed by environmental factors (Pong, 2013).

Osmolality can also be affected by low molecular species such as chlorine, sodium, and potassium present during the staining process, as in the Rose Bengal and fluorescein stains. The ferning patterns may result from electrolyte interactions of these macromolecules with polysaccharides and proteins (Friedman, 2010; Evans et al., 2012). This may explain why the current study failed to establish any significant relationship between HT and tear osmolality. The data spread further indicates that not all the study subjects that had a higher osmolality value reported improved DES symptom reduction. In conclusion, what remains clear is that HT use is negatively correlated with the DES symptoms in the postmenopausal women. This is contrary to the observation by Cumming and Mitchell (1997), who maintain that HT can alleviate the DES symptoms in persons with extreme symptoms.

#### **6.4. The OSDI and SANDE severity measurements**

The OSDI is designed as a disease-specific questionnaire, and is expected to record all the complaints of the dry-eye disease associated with the DES in postmenopausal women, such as irritation, burning, and a gritty feeling. Table 4 presents results of severity measurements after 38



months of HT treatment. The mean OSDI score was 1.5, which indicates moderate DES symptoms. At 36 months of treatment, there was no potential difference in tear osmolality and OSDI score. Thus, it appears from the research findings that estrogen-only HT treatment (Figure 1) on postmenopausal women does not significantly reduce the effects of DES. Healthcare providers should therefore inform their clients about the potential outcomes of HT use in the management of DES (Mitchell et al., 2002).

Similarly, in the test groups that used estrogen-only therapy, and in the estrogen and progesterone group, a small proportion of participants attained the normal tear osmolality of  $>307$  mOsm/L ( $p < 0.77$ ). As shown in Figures 2 and 3, participants that were treated with estrogen-only HT appears to have had more severe cases of DES than did subjects administered with estrogen and progesterone HT. On further evaluation of participant progress at 36 months, the results indicate that HT use in the test groups is not statistically related to tear osmolality and hence that the changes in DES severity were minimal ( $p < 0.48$ ;  $p < 0.12$ ). The study findings indicate that HT dose has no relationship with reduction in the DES symptom scores (Table. 7). These claims are supported by findings recorded from the SANDE questionnaires and the OSDI. The SANDE frequency was statistically insignificant when HT was used to treat the DES occurrence ( $r = -0.04$ ,  $p < 0.81$ ). SANDE severity did not show any relationship between HT medication and the severity of DES ( $r = 0.06$ ,  $p < 0.52$ ). Similarly, HT was non-significant related to ocular-surface disease progression (OSDI  $r = 0.54$ ,  $p < 0.79$ ).

HT appears to influence the functional and structural aspect of the eye when estrogen is used to contribute to increased risk of ocular surface disorders (Begley et al., 2003). Therefore, HT therapy offers an opportunity for further research into the flow and ebb of the sex hormones such as progesterone and estrogen. Commonly, the normal cycle exhibits low progesterone levels during the follicular phase. The subsequent surge of estrogen results in ovulation after the estrogen peak. The luteal phase exhibits increased progesterone levels while there reduced levels of estrogen during the same phase (Burton et al., 2004; Guthrie et al., 2000). Since hormone production is reduced during menopause, it can be assumed that the administration of estrogen-only HT may result in progesterone imbalance, contributing to adverse effects on the ocular surface, than when estrogen and progesterone HT is used (McCarty et al., 1998).

This is supported by the finding that subjects under estrogen and progesterone therapy showed a statistically significant reduction in DES severity after 36 months of treatment (M=50.78, SD =12.33). In addition, *post hoc* analysis shows that subjects who used <1mg/day HT dosage had a mean score of (M=61.22, SD = 25.37). These findings are significantly different from those for women who used >1mg/day of estrogen-only HT dosage (M = 74.171, SD = 23.45). There is a significant relation between HT use and DES in the current study, which shows the possible benefits of HT combination resulting from synergistic effects over the single therapy.

Most of the studies evaluating changes on the ocular surface during menopause and the possible effect of HT on these changes draw their findings from a various study subjects. Subjective dry eyes reduced with increased use of estrogen and progesterone therapies. The explanation for this visible change is still debatable, although central corneal thickness has been found to increase in postmenopausal women who use this HT combination. This fact suggests that progesterone may be relieving the DES symptoms (Nichols and Sinnott, 2006). Tomlinson et al. (2001) failed to establish a significant relationship between DES symptoms and tear film parameters after their study population was subjected to HT medication. The ocular symptoms and decline in DES severity happened at variable points throughout the research inquiry; thus, their information might have been recorded from a range of hormone variations during HT administration, as contrasting to hormone troughs and heights, as in the menstrual period.

### **6.5 Effect of HT use on dry eye**

SANDE analysis indicates a reduction in DES severity ( $p < 0.001$ ) and frequency ( $p < 0.037$ ) after using estrogen-and-progesterone-based HT. The severest test indicates an insignificant correlation in subjects that used estrogen therapy alone after the 36 month treatment period ( $p < 0.378$ ). 38% of the control group that did not use any HT reported increased DES symptoms. This means that the use of estrogen HT did not contribute to improve DES of tear osmolality as evaluated by the OSDI (Lemp et al., 2006).

Nonetheless, HT use (estrogen and progesterone) is associated with a considerably low SANDE score. A greater number of study participants report reduced DES symptoms in their ocular history. Even if the study groups showed significantly improved symptoms over the control group, HT use is likely to explain for changes in the SANDE scores and thus for improved DES.

In most cases, DES tends to increase with age. Although most of the larger-population studies on DES have used subjects above 50 years of age, the mechanisms that contribute to this difference are still unclear (Sullivan, 2004).

In addition, age and race differences do not contribute to any significant differences in the SANDE scores. This indicates that the effects of HT on DES are not influenced by such demographic factors (Table 1). Even if the effects of ethnicity or race on DES are indistinct, the limited data available in the literature argues that the occurrence of DES is higher in Asian and Hispanic women than in Caucasians (Schaumberg et al., 2003). The current finding that HT (estrogen-alone) has an insignificant effect on tear osmolality is consistent with other literature reports. For instance, Frankel and Ellis (1978), Tomlinson et al. (2001), Chalmers and Begley (2006), and Lemp et al. (2006) have reported no difference in tear break-up time and Schirmer's. Further, Tomlinson et al. (2001) have been unsuccessful to establish any association in turnover rate, volume between controls and HT users, evaporation rate, pre-rupture phase, or tear osmolality (Tomlinson et al., 2001).

## **6.6 Self-Assessment Questionnaire of the effect of HT on dry eye**

In the last ophthalmologist questionnaire survey, a physical exam and survey analysis were undertaken to assess DES symptom severity. The questionnaire assessed several scores related to mucoid secretion, burning, tearing, cornea, foreign-body sensation, and redness of the eyes (Table 7). Analysis findings indicate that women on HT had fewer DES complaints of these symptoms than the control group. While the exact mechanism of this relationship is still not clear, use of estrogen-alone in the current study indicates increased inflammation, reduced tear production, subsequent tear-film osmolality, and increased evaporation (Nicholas et al., 2006; Miller et al., 2004).

Although 38% of estrogen-only users compared to 21% of estrogen and progesterone users gave self-reports of increased dry eye in the last months of the treatment period ( $p < 0.01$ ), the OSDI and tear-osmolality values were not significantly different. Only 15% of the estrogen-and-progesterone-therapy users had DES, as indicated by an OSDI score of  $>13$ , which is the cut-off value for mild DES. The rate of DES from the questionnaire was much lower than the 55 - 82% reported in various studies that used self-reported questionnaires such as the contact-lens DES

questionnaire (Chalmers and Begley, 2006; Begley et al., 2002; Nicholas et al., 2006; Nicholas et al., 2005).

## **6.7 Ocular surface assessment after 36 month treatment**

Most women that report for HT treatment complain of ocular dryness and various DES signs. Proponents have argued for the effectiveness of HT in alleviating the DES symptoms. Guillon et al. (2011) argue that postmenopausal women with DES symptoms are likely to present with medical symptoms of DES after prolonged use of HT. Similar observations have been made by Guillon and Maissa (2005). HT use can modify the tear film and separate it into diverse layers marked with increased evaporation and destabilized stability (Glasson et al. 2006; Fonn 2007). However, the mechanism through which the dry-eye condition persists after HT use is unclear. The literature indicates increased tear-film instability and high osmolality (Baudouin 2007). Hyper-osmolality is currently recognized as the primary mechanism of ocular inflammation resulting in changed production of tears and altered release of the meibomian glands. In the current study, reports of DES complain may be attributed to altered lipid-layer thickness (Workshop 2007).

According to Pisella et al. (2001), high levels of hormone imbalance during HT use may contribute to overexpression of the human leukocyte antigen by the conjunctiva epithelium in hormone therapy users. The changes may further be mediated by oxygen free radicals that can contribute to cellular inflammation and alterations. In this study, analysis shows high mean rose-Bengal staining levels expressed in the study group. In line with the literature, inflammation and cytology changes are related to direct ocular surface irritation on the conjunctiva surface (Adar et al. 1997; Knop and Brewitt, 1992).

One hypothesis for increased ocular surface irritation after HT use is that it is perhaps linked to tear instability as a result of decreased goblet cells due to inflammation. Similar suggestions about the keratoconjunctivitis sicca have been made by a number of researchers Knop and Brewitt (1992) and Pisella et al. (2000). Inflammation appears to be more severe in the estrogen-only users than in estrogen and progesterone users, according to the current study. However, the expression of ocular surface severity from the OSDI, and of SANDE severity and SANDE frequency appear to be weak in the current study, which suggests low-grade inflammation in study groups under HT.

Both the estrogen-only and estrogen/progesterone users exhibited similarities in tolerance and intolerance to the ocular surface symptoms. The key difference was the time taken to observe the changes, with the estrogen and progesterone users taking a long time. Another explanation for the increased severity symptoms on the ocular surface is that HT causes structural alterations to the meibomian glands which result in tear instability and modified meibomian glands (Nichols and Sinnott 2006).

The current study also observed that the estrogen-only users and estrogen/progesterone users had a shorter tear break-up time compared to the control group ( $p < 0.0001$ ). High levels of osmolarity have been shown to be the pathogenic factor and marker for dry-eye disease. Studies on tear osmolarity and ocular surface symptoms in postmenopausal women under HT indicate that as osmolarity increases, DES symptoms become worse (Nichols and Sinnott 2006).

### **6.8. Tear function change in response to HT**

Postmenopausal women who take HT combination appear to have fewer complaints than women that do not receive any therapy. As evident from the Schirmer's test, the values were higher in women that were under HT for a period of 28 months. This may indicate that HT has beneficial effects on postmenopausal women with dry-eye syndrome. In line with the current study, lysozyme and IgA levels are said to increase with increased treatment duration. The inflammation of the meibomian gland also appears to reduce during the treatment, as evident from the absence of new patient complaints.

Sullivan et al. (1998) have shown that when estrogen-alone is administered to postmenopausal women for 2 years; there are no significant changes in the amount of protein in the tears. However, the use of estrogen and progesterone combination has a significantly high protein concentration and total protein in the animal (mice) models (Sullivan et al., 1998). The influence of androgens on lacrimal glands and changes to the gland's functions has been reported to be affected by gender, species, and time.

Compared to postmenopausal women whose hormone levels diminish with time, women in their early postmenopausal age have little tear function given their high levels of hormones. Despite this, other hormones such as testosterone have been reported to have a positive relationship with tear functions in postmenopausal women (Mathers et al., 1998). Estrogen HT has a negative effect on the development of dry-eye syndrome, an effect that appears to worsen as the ovarian

system starts to lose its functions. Besides, there was a mild Schirmer's test result as a result of estrogen and progesterone HT treatment.

Although the study could have used a placebo control group, the placebo could not have explained its effect during the trial period, as the average length of the study group exceeded 6 months. Even if more time was added to the placebo trial, the outcome may not have differed from the initial ratings of the study groups. As noted earlier, Schirmer's test and eye symptoms can relieve the symptoms of keratoconjunctivitis sicca if managed with  $17\beta$ -estradiol (Sator et al, 1998). The topical treatment, when compared with different tear substitute, has been reported to have a significant difference in the visual analogue score. However, the treatment route was via systemic administration of HT, and it therefore did not pass the blood-eye-barrier to have direct impact on the conjunctiva.

Rather than the oral estrogen administration, topical estrogen therapy is the factor that affects the symptoms of the DES. No topical medications were used by the patients that had DES at the beginning of the study, perhaps contributing to non-significant correlation between the estrogen-only therapy and the reduction of the dry-eye symptoms. Even so, there is a positive effect of the improved meibomian gland function in both patients using estrogen-only therapy and those under estrogen and progesterone therapies. For its androgen effect, the meibomian gland targets the production of the oil component of the tears.

Some of these properties include an improved secretory component of the eye and IgA production (Sullivan et al., 1988). In the current study, no significant difference was found of reduction in the severity of DES symptoms in the estrogen-only HT users. Framingham study reported that the use of estrogen therapy is inversely related to increased cases of nuclear opacities and DES symptoms. In this case, the study duration was more than 10 years and participants had sixty percent risk reduction compared to non-estrogen users. The Beaver Dam eye study, subjects reported reduced lens opacities with estrogen use, further demonstrating the hormonal effects on the dry-eye syndromes (Klein et al., 1994). The Melton eye study found that the use of estrogen therapies has no effect on cortical opacity (Thompson et al., 1996). The Blue Mountains research found that women over 65 report fewer cortical lens opacities than non-estrogen users (Cumming et al., 1997). However, McCarty et al. (1999) failed to establish any

relationship between HT and dry-eye disease in their Melbourne Visual Impairment Project study.

In the future, a large sample may be used to evaluate the current findings, preferably with a longer treatment period of more than two years. For example, with a longer study duration, the However, additional evaluations need to be made to determine whether postmenopausal use of estrogen contributes to reduced dry-eye symptoms in the long-term. In another study,

## **6.9. Study Limitations**

The first limitation is that no control was made for the different dry-eye diseases or types, environmental stress, climate, diurnal variations, and the individual lifestyle changes and patterns. Moreover, researchers Caulfield et al. (1999) pointed out that the DES severity measurements differ widely, and they therefore emphasizes the importance of measuring DES three times to obtain a more reliable figure. The current study also performed a single-eye osmolarity test on each eye at the time of the visit. Future studies that perform three successful measurements on each eye may therefore yield more reliable results.

The current study was also limited to examining objective symptoms of DES marker—symptoms, severity that have been shown to exhibit DES specificity and sensitivity in subjects using HT. However, the study did not undertake a comprehensive clinical analysis of dry eye sub-type status during the study visits. Finally, the study did not consider the epidemiological aspect across different ethnic or racial backgrounds to assess its possible effect on DES severity among women who received HT during the treatment duration.

## **7. Conclusion:**

Evidence for the relationship between use of HT and reduction of DES in postmenopausal women continues to be elusive. This is because DES has the potential to impair normal functioning. However, a significant relationship between DES and sex hormones exist with mild, moderate or severe symptoms in postmenopausal women.

Besides, the statistical analysis in the present study showed significant variation in severity levels of DES. This clearly indicates that women under estrogen HT are at higher risk than women under estrogen-and-progesterone HT. The presence of mild levels of DES among women who are not under HT clearly indicates that women under HT need to reassess their acceptance of menopausal treatments to avoid ocular surface discomforts.

Duration of HT administration and dosage levels of HT also play significant roles in increasing the risk of DES.

The study has its own strengths. It is novel for its large sample size, near-equal distribution of sample (under HT and not under HT), and combination of IDEEL and OSDI.



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# Questionnaire

## Dry-eye Questionnaire Survey (Adapted from Tear Lab Corp, 2013)

### Dear participant,

Please note that you are answering a questionnaire whose data will be used in a cross-sectional study which will compare the presence and occurrence of dry-eye syndrome in postmenopausal women who are under HT and not under HT. The information (personal or otherwise) provided by you is highly confidential and strictly used for research studies. By taking this survey, you provide us with voluntary consent to participate in this study. Please read each question carefully and answer to the best of your capability. Thank you.

Patient Name or ID:

Date:

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### 1. Have you ever been diagnosed with Dry-eye disease or Ocular-surface disease?

Yes       No       When?

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### 2. Do you have any of the following symptoms?

Blurry vision

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Redness

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Burning

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Itching

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Light sensitivity

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Excess tearing/ watering eyes

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Tired eyes, eye fatigue

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Stringy mucus in or around the eyes

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Foreign-body sensation

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Contact lens discomfort

IF yes, please tick the correct answer:  constantly  often  sometimes  never

Scratchy feeling of sand or grit in the eye

IF yes, please tick the correct answer:  constantly  often  sometimes  never

### 3. Have you had any of the following surgeries?

Cataract:  Y  N

Glaucoma:  Y  N

Refractive Surgery:  Y  N

### 4. Do you use?

Contact lenses

IF yes, please tick the correct answer:  constantly  often  sometimes  never

OTC eye drops such as artificial tears

IF yes, please answer: constantly often sometimes never

Rx eye drops for Dry-eye syndrome (e.g., Restasis)

IF yes, please tick the correct answer: constantly often sometimes never

Rx eye drops for Glaucoma (e.g., Xalatan, Timolol)

IF yes, please tick the correct answer: constantly often sometimes never

Rx eye drops for Allergy (e.g., anti-inflammatory, antihistamine)

IF yes, please tick the correct answer: constantly often sometimes never

Nutritional supplements (e.g., flaxseed oil, omega-3)

IF yes, please tick the correct answer: constantly often sometimes never

**5. Are your symptoms related to the following environmental conditions?**

Windy conditions

IF yes, please tick the correct answer: constantly often sometimes never

Places with low humidity (e.g., airplanes/hospital)

IF yes, please tick the correct answer: constantly often sometimes never

Areas that are air conditioned/heated

IF yes, please tick the correct answer: constantly often sometimes never

**6. Are you taking any of the following medications?**

Hormone-replacement therapy (estrogen)

IF yes, please tick the correct answer: constantly often sometimes never

Hormone-replacement therapy (estrogen and progesterone)

IF yes, please tick the correct answer: constantly often sometimes never

Antihistamines/decongestants

IF yes, please tick the correct answer: constantly often sometimes never

Antidepressant or anti-anxiety

IF yes, please tick the correct answer: constantly often sometimes never

Oral corticosteroids

IF yes, please tick the correct answer: constantly often sometimes never

Antihypertensives (e.g. diuretic, beta-blocker)

IF yes, please tick the correct answer: constantly often sometimes never

Accutane or other oral treatment for acne

IF yes, please tick the correct answer: constantly often sometimes never

**7. Have you ever had punctal occlusion?** Y N

## **Acknowledgement**

Primarily, I would like to thank my supervisor Prof: M. E. Hammadeh with my kind respect for his support, trust, help and guidance in all the period of my present research.

I would like to thank my lovely parents for their absolute trust, any kind of support and their soothing words which made me continue in all my life since I born.

Finally, I would like to give my precious thank to my family and my lovely wife. Thanks to her patients and invaluable supports.