EVALUATION OF MACULA AND RETINAL NERVE FIBER LAYER THICKNESS IN POSTMENOPAUSAL WOMEN WITH HORMONE REPLACEMENT THERAPY

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ABSTRAK

Pengenalan

Menopaus adalah fasa di mana ovari secara semula jadi tidak lagi menghasilkan hormon iaitu estrogen dan progesteron. Menopaus selalu dikaitkan dengan pelbagai tanda tanda sistemik termasuklah simptom vasomotor, genitourinari, kesukaran tidur dan lain lain lagi. Selain itu, menopaus juga melibatkan perubahan pada struktur mata. Penyusutan estrogen menyebabkan pengaliran darah di dalam mata terganggu kerana estrogen mempunyai kesan pada tona salur darah: salah satu penyumbang kepada terjadinya penyakit seperti 'age related macular degeneration (ARMD)' dan risiko glaukoma. Terapi gantian hormon merupakan rawatan terbaik untuk simptom menopaus kerana ia mengurangkan frekuensi dan tahap teruk ketidakselasaan yang disebabkan pengurangan estrogen. Pada masa yang sama, rawatan ini juga membantu dalam mengekalkan fungsi dan struktur mata di kalangan wanita menopaus.

Objektif

Matlamat kajian ini adalah untuk menilai ketebalan makula dan lapisan fiber saraf retina di kalangan wanita yang telah menopaus dengan terapi gantian hormon secara oral dan tanpa terapi gantian hormon dan juga mengkaji korelasi di antara durasi rawatan terapi gantian hormon secara oral dengan ketebalan makula dan lapisan fiber saraf retina.

Kaedah Kajian

Kajian ini merupakan kajian keratan rentas yang dikendalikan di Hospital Universiti Sains Malaysia dan Hospital Raja Perempuan Zainab II, Kelantan di antara Julai 2019 sehingga November 2020. Sejumlah 100 wanita menopaus yang memenuhi kriteria pemilihan telah

dipilih berdasarkan persampelan bukan kebarangkalian dan dibahagikan kepada dua kumpulan iaitu kumpulan wanita menopaus dengan terapi gantian hormon secara oral dan kumpulan wanita menopaus tanpa terapi gantian hormon. Paras ketebalan makula dan ketebalan lapisan fiber saraf retina diambil menggunakan mesin OCT Hiedelberg Spectralis oleh pegawai terlatih tanpa mengetahui kumpulan kajian. Peserta kajian hanya perlu datang sekali sahaja untuk persampelan data. Analisa statistik dijalankan menggunakan SPSS versi 26.0.

Keputusan

Tiada perbezaan purata yang ketara dalam purata ketebalan makula di antara kumpulan wanita yang telah menopaus dengan terapi gantian hormon secara oral dan tanpa terapi gantian hormon kecuali untuk kawasan `superior outer macula' ($p=0.042$) dan `temporal outer macula' ($p=0.007$). Sementara itu, untuk purata ketebalan lapisan fiber saraf retina, tiada perbezaan purata yang ketara di kalangan dua kumpulan ini kecuali untuk kawasan `temporal' ($p=0.044$), `temporal superior' ($p=0.002$) dan `temporal inferior' ($p=0.033$). Tiada korelasi yang ketara di antara ketebalan makula dan ketebalan lapisan fiber saraf retina dengan durasi rawatan terapi gantian hormon secara oral (semua $p>0.050$).

Kesimpulan

Kajian ini menyimpulkan bahawa terapi gantian hormon di kalangan wanita menopaus menunjukkan kesan positif pada struktur mata terutamanya pada ketebalan makula (khususnya pada `superior outer macula' dan `temporal outer macula') dan ketebalan lapisan fiber saraf retina (khususnya pada `temporal', `temporal superior' dan `temporal inferior'). Oleh itu, penggunaan terapi gantian hormon dalam wanita menopaus memberi kesan yang baik untuk struktur mata.

ABSTRACT

Introduction

Menopause is a state of natural ovarian senescence with accompanying estrogen deficiency. It is associated with various systemic manifestations due to estrogen deficiency including vasomotor, genitourinary, sleep disturbance and others. Besides, menopause is also associated with ocular changes. Declination of estrogen causing impaired ocular blood flow as estrogen have the ability to influence the vascular tone and blood flow in organs and tissues; the impaired ocular blood flow is contributing factor in aetiology and progression of glaucoma and age related macular degeneration (ARMD). Hormone therapy is used as the most effective treatment for menopausal symptoms because it reduces the frequency and discomfort causing by declination of estrogen in the body. In the meantime, it also has the advantages towards eyes structures itself.

Objective

To evaluate macula and retinal nerve fiber layer thickness in postmenopausal women with and without oral hormone replacement therapy (HRT) and to correlate between duration of oral HRT treatment and macula and RNFL thickness.

Methods

This is a cross sectional study conducted at Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II between July 2019 until November 2020. A total of 100 menopause women who fulfilled selection criteria were selected using non-probability sampling method. They were divided into two groups: Group 1: postmenopausal women with oral HRT treatment

and Group 2: postmenopausal women without HRT treatment. The macula and retinal nerve fiber layer thickness were taken using Hiedelberg Spectralis OCT machine for both groups by blinded trained personnel. The participants came only once during the study period. Statistical analysis was performed using SPSS version 26.0.

Results

There were no significant mean differences of mean macula thickness between postmenopausal women with and without oral HRT except for superior outer macula ($p=0.042$) and temporal outer macula ($p=0.007$). Meanwhile, for RNFL thickness, there were no significant mean differences of mean RNFL thickness between postmenopausal women with and without oral HRT except for total temporal ($p=0.044$), temporal superior ($p=0.002$) and temporal inferior ($p=0.033$). There was no significant correlation between macular thickness and RNFL thickness with duration of oral HRT treatment (all $p>0.050$).

Conclusion

HRT usage demonstrated positive structural changes on the macula thickness (specifically on superior outer macula and temporal outer macula) and RNFL thickness (over the temporal, temporal superior and temporal inferior). Hence, the usage of HRT is benefited to the ocular structures of postmenopausal women.

CHAPTER 1
INTRODUCTION

1.1 Introduction

One of the most important part in woman's life is menopause. It refers to a state of ovarian failure and ovarian destruction/removal with accompanying estrogen deficiency (Yasuda, 2010). It can be diagnosed either by clinical criteria which include age around menopause (around 50 years), no periods for 12 months and presence of menopausal symptoms (all 3 need not be present for a diagnosis) and by laboratory criteria which is FSH level >35mg/ml.

Menopause is associated with various systemic manifestations due to estrogen deficiency; examples include vasomotor symptoms like hot flushes, night sweats, vaginal symptoms: vaginal dryness, lower urinary tract symptoms: dysuria, frequency of micturition, nocturia and others. Besides that, menopause is also associated with ocular changes such as effects on tear film, intraocular pressure, lens, ocular blood flow and retina (Altintas, 2004).

Hormone therapy (HT) is used as the most effective treatment for menopausal symptoms but in Malaysia, only about 10% of postmenopausal women are taking hormone therapy (HT), while 30-40% are on herbal remedies. This happens because menopausal women are still apprehensive and anxious about its continued use, contributed by cultural practices, social mores, misinformation through media, and differing practices of medical personnel based on inconsistent scientific data (Yasuda, 2010).

Background

1.2.1 Menopause

Menopause is a natural stage in women's life and is part of the ageing process. It occurs when a woman stops having menses as she reaches the end of her natural reproductive life. Clinically, menopause is defined as the permanent cessation of menstruation due to loss of ovarian follicular function and usually diagnosed after 12 months of amenorrhea. Globally, the average age of menopause is around 51 years old and in Malaysia, the mean menopausal age is 50.7 years old (Abdullah et al., 2017). In Malaysia, more women are living longer with a current average life span of 75.2 years indicates that nearly a third of women's life is now spent on menopause (Dhillon et al., 2006).

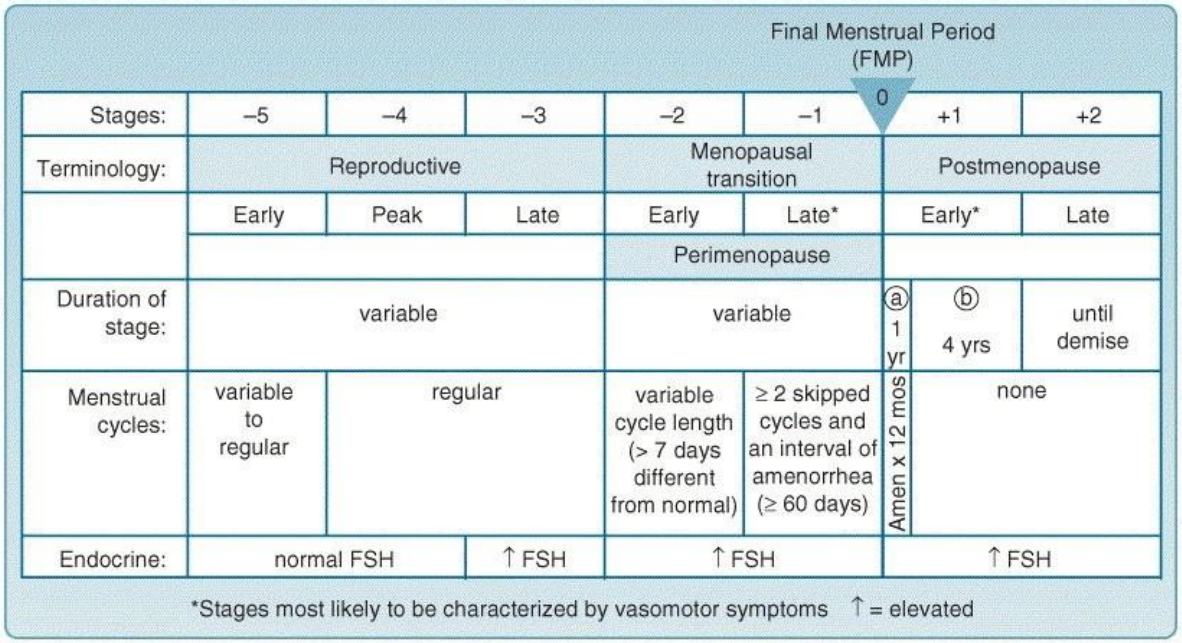
Natural menopause occurs when the ovaries naturally decrease their production of sex hormones that is estrogen and progesterone whereas in medical menopause, it refers to menopause occurring as a result of permanent damage to both ovaries in women following either chemotherapy or radiotherapy. On the other hand, surgical menopause is when menopause occurring as a result of surgical removal of both ovaries in a woman (Yasuda, 2010).

A menopause terminology and staging system has been developed to provide a consistent way of describing the transition in midlife women (Figure 1.1 and Figure 1.2). The early menopausal transition stage is characterised by elevated follicle-stimulating hormone (FSH) and a variable cycle length (more than 7 days deviation from normal). FSH levels increase in response to the absence of estrogen and progesterone that would normally provide negative feedback to the hypothalamus to inhibit FSH secretion. Clinically, this period can be divided

into pre-menopause, which is often used ambiguously either to refer to the one or two years immediately before the menopause or to refer to the whole of the reproductive period prior to the menopause. The WHO Scientific Group on Research on the Menopause in the 1990s recommended that the term to be used consistently in the latter sense. This encompass the entire reproductive period up to the final menstrual period and perimenopause stage, the period immediately prior to the menopause (when the endocrinological, biological and clinical features of approaching menopause commence) and the first year after menopause.

The late menopausal transition stage is characterised by two or more skipped menstrual cycles and an interval of amenorrhea (at least 60 days) as well as elevated FSH levels. The post-menopausal is also divided into stages. The early post-menopause stage is defined as 5 years since the final menstrual period and subdivided into two segments: 1) the first 12 months after the final menstrual period and 2) the next 4 years. The late menopause stage begins with year 6 after the final menstrual period and continues through the remaining life span.

Figure 1.1 Stages of normal reproductive ageing in women.



*The final menstrual period (FMP)is the time in a woman's life when she has missed 12 consecutive menstrual period (amenorrhea) and considered as early postmenopausal or late postmenopausal stages. Before permanent cessation of menstrual cycles, women vary in the duration of their cycles during the stage known as perimenopause (Adapted from Soules et al., 2001).

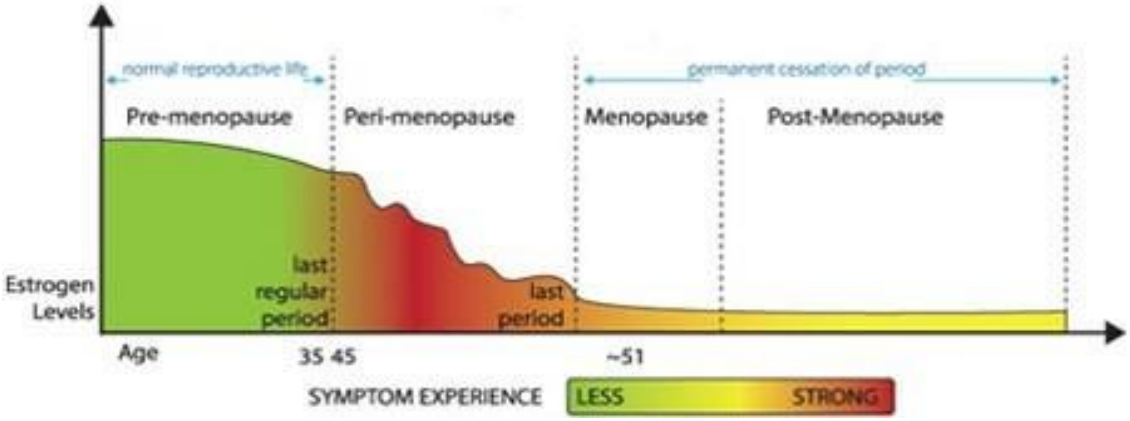


Figure 1.2 Transition period from pre-menopause to post-menopause stage in relation to estrogen level (Adapted from www.live-cool.com).

The most important hormone in menopausal endocrinology is estradiol, which has multiple target tissues including the brain, breasts, bone, cardiovascular system, liver, uterus and vagina. Besides estradiol, during menstrual life, the ovaries make androgens and progesterone. It is the ovarian dominant follicle and corpus luteum produce the most potent of the three human estrogens, 17 estradiol (E2). The peripheral aromatisation of androstenedione in adipose and muscle and the metabolism of E2 produce the second most abundant estrogen, estrone (E1). Small amounts of estrone are also produced by the ovary and adrenal glands. The premenopausal ratio of circulating estradiol to estrone of greater than 1 reverse after menopause, with estrone becoming the dominant circulating estrogen. Estriol (E3) is the weakest of the three human estrogens. During the childbearing years, the ovary makes inhibin,

which inhibits the release of follicle stimulating hormone (FSH). Inhibin is no longer made in the postmenopausal years, thus allowing for the rises in FSH seen with menopause. Progesterone is produced primarily by the corpus luteum and is the only naturally occurring progestogen. Progesterone opposes estrogen's actions and converts proliferative endometrium into a secretory endometrium in preparation for implantation and pregnancy, with levels decreasing during the menopause transition. Chemically altered, predominantly plant based progestins are synthetically manufactured to oppose the effects of estrogen in pharmacologic hormone therapy (HT) (Andrea L. Sikon, Holly L. Thacker, (2007). General Gynecology: Chapter 16. Menopause, page 383-384.).

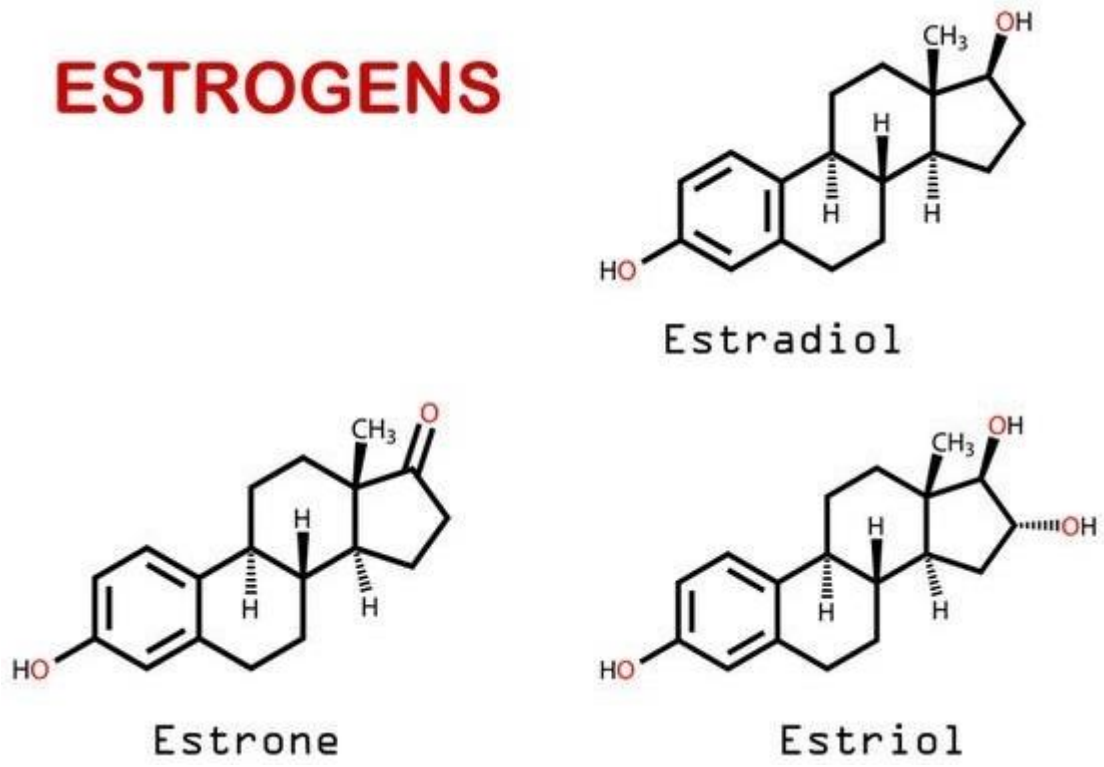


Figure 1.3 Chemical structure of estradiol, estrone and estriol (Adapted from holistichealingnews.com).

1.2.2 Effects of Menopause

1.2.2.1 Systemic Effects

As a woman ages, the ovaries progressively fail to produce estrogen. This failure often begins in the late 30s, and most women experience near-complete loss of production of estrogen by their mid-50s. The transition from normal ovarian function to ovarian failure is described as the menopausal transition. Although for many women menopause is asymptomatic and associated with little disruption of normal life and well-being, many women experience symptoms—sometimes severe and disabling—that considerably affect their quality of life.

The top 3 most common menopausal symptoms experienced by Malaysian women are sleeplessness, headache and reduced sex drive. Nevertheless, the experience of menopausal symptoms can be quite varied in different populations (Yasuda, 2010).

In one study conducted by Dhillon et al., (2006) among postmenopausal women in Kelantan, night sweats (53%), headache (49.4%) and hot flushes (44.8%) were the typical vasomotor symptoms, whereas mood swings (51%), sleep problems (45.1%), loneliness (41.1%), anxiety (39.8%) and crying spells (33.4%) were the main psychological symptoms. Uro-genital symptoms such as vaginal discomfort (45.7%), occasional stress incontinence (40%), weak bladder control (24%) and urinary tract infection (19.3%) were also reported among menopausal women living in Kelantan.

Besides, as women lose support of estrogen, they are at increased risk for developing osteoporosis and heart disease. Estrogen declines associated with menopause increase bone remodelling, leading to an imbalance between bone formation and bone resorption. This

increase in bone remodelling persists over several years and becomes associated with an increased rate of bone loss. Several mechanisms likely underlie bone loss at menopause. Estrogen deficiency leads to T-cell activation and studies in mice have shown that ovariectomy does not induce bone loss in mice depleted of T-cells with T-cell antibodies. Activation of T-cells by ovariectomy increases T-cell production of TNF, a cytokine that stimulates osteoclast formation by potentiating the activity of RANKL (receptor activator of nuclear factor-kappa B), and by promoting production of RANKL by osteoblast cells (Maryfran R.Sowers , 2003) .

In a study conducted by Recker et al., (2000) a longitudinal study of 75 women with a mean age of 46 years old at baseline, who were followed for 9.5 years, showed that bone loss began about 2–3 years before menopause and ended 3–4 years after the last menses. The total loss in the spine and femoral neck was 10.5% and 5.3%, respectively, over the menopausal period. Results suggested that menopausal bone loss is a composite of loss caused by estrogen deprivation and age for the hip, but estrogen deprivation alone for the spine. This will eventually lead to osteoporotic fractures.

In most developed countries, cardiovascular disease (CVD) is the leading cause of mortality and morbidity in women aged over 50 years. CVD and cerebrovascular disease account for the majority of deaths in postmenopausal women (75–76%), a significantly higher proportion than breast cancer (6–8%). Menopause negatively impacts upon many traditional risk factors for CVD, including changes in body fat distribution from a gynoid to an android pattern, reduced glucose tolerance, abnormal plasma lipids, increased blood pressure, increased sympathetic tone, endothelial dysfunction and vascular inflammation (Rosano et al., 2007). Increases in body fat in both men and women are associated with many detrimental effects. In particular,

increased body weight and obesity are associated with reduced insulin sensitivity and increased blood pressure, changes which are enhanced in postmenopausal women by estrogen deficiency. Compared with premenopausal women, postmenopausal women have significantly higher insulin resistance. Insulin resistance leads to high levels of circulating insulin, causing sodium and fluid retention, leading to high blood pressure and congestive heart failure. In postmenopausal women, treatment of arterial hypertension and glucose intolerance should be priorities.

1.2.2.2 Ocular Effects

Significant gender-based differences in the incidence of age-related macular degeneration (ARMD), primary open-angle glaucoma(POAG), nuclear cataract and idiopathic macular hole (Zetterberg and Celojevic, 2015) (Nuzzi et al., 2018) raise the possibility that estrogens may have a direct effect on the eye.

In a study conducted by O Altintas et al., (2004) they found out that age-induced changes on quality and amount of tear, IOP and retrobulbar blood flow are intensified by the menopause and that it may be possible to decrease the menopausal effects on these parameters by HRT. Diffuse fibrosis and atrophy of the lacrimal ducts – often found during the menopause – may contribute to the development of KCS (Conrady et al., 2016). It has also been reported that meibomian glands contain androgen, estrogen and progesterone receptor messenger RNA and protein within acinar epithelial cells, that they express messenger RNAs for both type-1 and -2 5·-reductase and respond to an androgen precursor by increasing production and release of lipids (Wickham et al., 2000). The same study suggested that the meibomian gland is an androgen target organ and that androgens control meibomian gland function, regulate the quality and/or quantity of lipids produced by this tissue, and promote the formation of the tear

film lipid layer. It has been hypothesized that androgen deficiency may be associated with meibomian gland dysfunction and an increase in the signs and symptoms of evaporative dry eye. Serum levels of total androgens decline in both sexes during aging and the menopause, develop lower levels of estrogen thus increased appearance of meibomian gland dysfunction and dry eye. The action of estrogen could also be explained by stimulation of nitric oxide synthase (NOS) and its vasodilatory effect, which may act not only on blood vessels but also on the lacrimal ducts (Goh et al., 1995).

They have also discovered that IOP in postmenopausal women was found to be higher than in menstruating women. This result tailors with several studies reporting the mean IOP in postmenopausal women to be higher than in age matched, still menstruating controls (Ebeige JA et.al, 2011; SRP Panchami et.al, 2013). The physiological mechanisms responsible for the increase of IOP in postmenopausal women are not fully known. IOP is maintained as a result of a balance between secretion of aqueous humor by the ciliary process and reabsorption or outflow of aqueous through the trabecular meshwork into Schlemm's canal and then through collecting channels to scleral veins via conventional pathway. These structures contain abundant nitric oxide synthase (NOS). It is postulated that trabecular meshwork cells have many characteristics in common with smooth muscle cells and that they contract in the presence of cholinergic agonists but relax with NO(nitric oxide) agonists (Goh et al., 1995) and responsible for regulating ocular outflow and IOP. In addition to altering the contractile response of the muscle light-chain kinase pathway in smooth muscle, NO causes a slight inhibition of aqueous secretion in nonpigmented ciliary epithelium by altering Na,K-ATPase activity (Ellis D, Nathanson JA, 1998). Presence of estrogen causes an increase in endothelium based constitutive NOS. Both NO and estrogen have been shown to play a critical role in the control of IOP by altering secretion and outflow of aqueous.

There are a few studies that correlate role of estrogen in pathogenesis of primary open angle glaucoma (POAG). The analysis of various studies showed that the factors modulating the duration of exposure to estrogen seem to influence the risk of developing a POAG: prolonged exposure to estrogen, such as that occurring in women entering menopause after age 54, (Pasquale and Kang, 2011) leads to a lower risk of developing POAG. On the contrary, all the conditions reducing the duration of exposure to estrogen such as the age at menarche over 13 years (Lee et al., 2003), bilateral ovariectomy before age of 43 (Vajaranant et al., 2014), and spontaneous menopause before age of 45 (Vingerling et al., 2001), increase the risk of developing POAG.

Chen et al., (2018) studied the role of estrogen on the regulation of IOP and consequently, on the status of retinal ganglion cells and evaluating the effect of the lack of aromatase, an enzyme necessary for the production of estrogens. In this study both wild type and aromatase knockout mice were compared (based on age and sex). The knockout females showed significantly higher IOP levels compared to wild type females of the same age and significantly lower RGC levels. These results further confirm the role of estrogens in regulating IOP, reducing it and ensuring optimal RGC status.

Besides, incidence of cataract is much higher in postmenopausal women as compared to males of similar age groups and sex hormones has significant role in prevention of cataract formation in postmenopausal women (Younan et al., 2002). Support for the biologic plausibility of an estrogen conferred protection against cataract has also come from a series of human and animal model studies of cataract and its etiology. First, α-estrogen receptor messenger RNA (mRNA) has been detected in human lens epithelial cells, suggesting a possible mechanism for a direct estrogen effect on the lens. Second, estrogen has been shown to protect against transforming

growth factor β (TGFβ)-induced cataract in a rat model of cataractogenesis. With the use of animal models, TGFβ has been shown to induce morphologic and molecular features of human subcapsular cataract and cortical cataract (Hales et al., 1997). Exposure to estrogen inhibits TGFβ-induced cataractous changes. Additional studies using this rat model suggest a correlation between the expression of the α-estrogen receptor in the lens epithelium and the ability of estrogen to protect against TGFβ-induced cataract.

Ogueta et al., (1999) reported for the first time the presence and localization of α -type estrogen receptors in the epithelia of several ocular tissues such as retina, lens and ciliary body (non-pigmented). Subsequently, Munaut et al., (2001) demonstrated the presence of α -type estrogen receptors and ß-type estrogen receptors and suggested that the sex steroid hormone axis may play a role in the pathogenesis of certain ocular diseases. Analysis using immune chemical localisation was done in neural retina, retinal pigment epithelium and choroid layer to demonstrate the presence of these receptors. They found out that α- type receptor was more widespread in the neural retina and its localisation correlated with previously performed analysis on human tissue, with more intensity in the ganglion cell layer whereas and ß-type receptors were mainly observed in the ganglion cell layer of the retina and mild and inconsistent in choroid. Distribution of estrogen receptor in the retinal layers is shown in Figure 1.4.

ER α ER ß Negative control

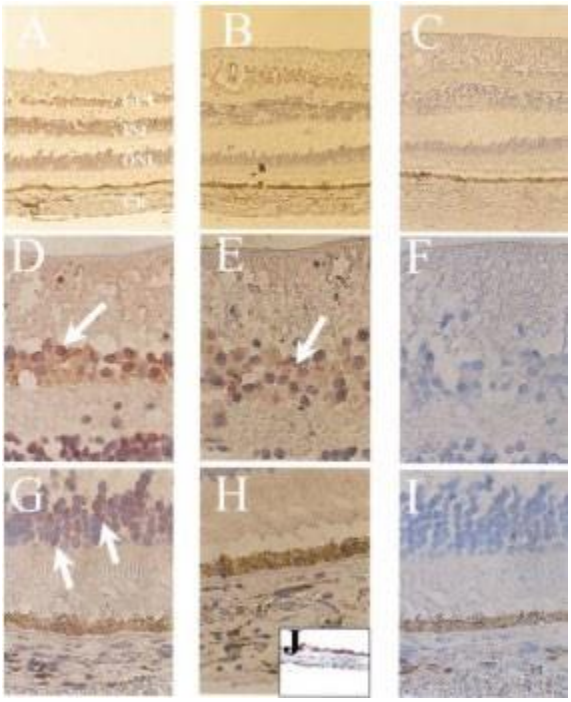


Figure 1.4 Distribution of estrogen receptor in the retinal layers. (A, B, C : low magnification x 100, of retinal- choroid layer) , (D, E, F : higher magnification x 400, focused on ganglion cell layer) , (G, H, I : focused on choroid-RPE complex) (Adapted from Munaut et al., 2001)

There is another study on mice that have identified differences in retinal structure between males and females and found that, as measured by multifocal electroretinography (mfERG), retinal function is better in females of reproductive age than in males and older females (Chaychi et al., 2015). Similar mfERG studies on humans found a statistically significant difference in neuroretinal function between men and women below 50 years, but not after this age; in addition, neuroretinal function was lowest in women who received a hysterectomy during reproductive age, with subsequent iatrogenic-induced menopause. These findings

suggest that the estrogenic cycle has a beneficial effect on neuroretinal function and that estrogens may have a protective role (Ozawa et al., 2014).

The most common maculopathy in ageing population is age-related macular degeneration (ARMD). It is chronic, progressive eye disorder that mainly affects people over the age of 50. It is the leading cause of blindness in Western world in people over 60 years and third most common cause globally after cataract and glaucoma (Mitchell and Bradley, 2006). Histological hallmarks are degeneration of the RPE, Bruch's membrane, and the choriocapillaris, resulting in photoreceptor damage and death.

The researchers suggested that oxidative stress plays an important role in the degeneration of the retinal epithelium. In women, the antioxidant properties which is in estrogen may have a protective role against oxidative stress on retina and thus helps promote the survival of the retinal epithelium. In post-menopausal women, due to declination of estrogen and directly less antioxidant effects increases the oxidative stress on the retina and makes them more prone to get AMD.

Another potential effects of estrogen is protection against macular hole. Although in some cases the cause can be identified, e.g. contusive trauma, cystoid macular edema, or diabetes, it is idiopathic in the majority and thought to be due to circumferential vitreoretinal contraction. Macular hole affects women far more often than men. With the sudden drop in estrogen production after menopause, it lost the effects to stimulate the synthesis of collagen and hyaluronic acid in the eye , posing the retina to a higher risk than that for men, in whom estrogen levels are generally low throughout life and do not change abruptly. Current evidence indicates

strong correlation between macular hole and female sex and postmenopausal age, as demonstrated by various studies (Nuzzi et al., 2018).

1.2.2.2.1. Retinal Nerve Fiber Layer Thickness in postmenopausal women

Retina nerve fiber layer (RNFL) is the innermost layer of retina consists of the axons of the ganglion neurons coursing on the vitreal surface of the retina to the optic disc. The RNFL exit the eye through the lamina cribrosa as optic nerve.

Based on few studies done for difference of RNFL thickness between gender, most of it showed no obvious difference between male and female. The mean RNFL thickness for the normal population is 97.3 ± 9.6 µm (Alasil et al., 2013 ; Cubuk et al., 2016).

There is limited study on comparison of RNFL thickness between reproductive and post-menopausal women. Ataş et al., (2014) conducted a study on evaluation of the macula, retinal nerve fiber layer and choroid thickness in postmenopausal women and reproductive age women using spectral domain optical coherence tomography. Peripapillary RNFL thickness parameters, macular thickness and choroidal thickness were evaluated. According to this study, no significant differences was found between the post-menopausal women and control group regarding all the peripapillary RNFL thickness parameters.

1.2.2.2.2. Macula Thickness in postmenopausal women

Macula is the vital structure of human's eyes and most sensitive part in the center of the retina. It is responsible for central vision and color vision due to abundance of photoreceptors. Any disease related to macular disturbance resulted in visual disruption and affecting the quality of life.

Several studies done showed that there is a difference in macular thickness between males and females (Adhi et al., 2012 ; Wagner-Schuman et al., 2011) in which male gender was associated with greater foveal and mean macular thickness compared to females. Therefore, it can be hypothesized that the disparity of macular thickness between males and females can be due to influence of sex hormones. The normal macular thickness in adult ranges from 203 μm to 335 μm (Pokharel et al., 2016).

So far, there is limited data on macular thickness comparing between post-menopausal women to reproductive age group women to assess whether there is an effect of female reproductive hormone on macula thickness. Ataş et al., (2014) conducted study about macular thickness and choroidal thickness in postmenopausal women and reproductive age women using SD-OCT. They found that superior inner macula, temporal inner macula, inferior inner macula, nasal inner macula, inferior outer macula and choroid thickness were significantly thinner in postmenopausal study group than healthy reproductive age control group. After adjusting for age, only choroid thickness was significantly thinner in the postmenopausal group than controls.

1.2.3 Treatment of Menopause

1.2.3.1 Hormone Replacement Therapy (HRT)

Hormone replacement therapy is one of the treatment options for treating menopausal symptoms besides some other alternative treatment namely acupuncture, herbal remedies for examples evening prime rose oil, ginseng and gingko biloba extract.

It should only be recommended when there is a definite and clear indication. Indications can be classified as: i) presence of symptoms ii) the need for prevention of osteoporosis. Symptoms in relation to menopause include vasomotor symptoms (hot flushes and/or sweating), joint and muscle aches and pains, sleeplessness and other sleep disturbances, depressed mood, uro-genital symptoms, particularly vaginal dryness, sexual dysfunction, including decreased libido. HRT also is been used as one of the first-line choices, for the prevention of bone loss in postmenopausal women as they have increased risk for fractures.

Hormone therapy refers to the use of estrogens (ET), estrogens plus progestins (EPT) and androgens (AT). EPT is synonymous with the term 'hormone replacement therapy' while ET is synonymous with the term 'estrogen replacement therapy'. Standard dose of ET & EPT refers to dose of estrogen in the preparation : either conjugated equine estrogen 0.625mg or estradiol valerate 2.0mg whereas low dose ET & EPT refers to dose of estrogen in the preparation : either conjugated equine estrogen 0.3mg or 0.45mg or estradiol valerate 1.0mg.

Examples of hormone therapy in Malaysia include estrogen therapy only i.e. Premarin: conjugated equine estrogen (0.3mg/0.625mg) and Progynova: estradiol valerate (1.0mg/2.0mg). For estrogen progestin therapy, which is indicated in woman with intact uterus,

is to provide endometrial protection from unopposed estrogen and the occurrence of endometrial hyperplasia and carcinoma. Example of EPT include Progyluton : 11 pills estradiol valerate 2.0mg with 10 pills estradiol valerate 2.0mg and norgestrel 500mcg, Femostan 1/10 : 1 tab (estradiol 1mg) OD for 14 days follow by 1 tab (estradiol 1mg & dydrogesterone 10mg) for remaining 14 days. Hormone therapy is available in the form of oral and transdermal (patch, gel, cream and implant). The dose, regime and mode of administration of HT need to be individualized. Older postmenopausal women generally require lower doses than younger women and transdermal routes have the advantage of avoiding the first-pass liver effect, and may be more favourable than oral routes, especially with regards to venous thrombolic event and cardiovascular risks.

1.2.4 HRT and Its Effects on Ocular Structures

There is evidence-based research that estrogen metabolism has a vital role in the pathogenesis of primary open-angle glaucoma (POAG). In a study done by Vingerling et al., (2001) showed that among women ≥ 55 years, early age of menopause (≤ 45 years) was associated with an increased risk of POAG. Meanwhile for women at later age of onset of menopause (≥ 54 years) was associated with a decreased risk of POAG. The similar study also found that in women compared with never use of HRT, current use of estrogen with progestin was associated with a reduced risk of POAG characterised by intraocular pressure >21 mm Hg before visual field loss.

Another study by Affinito et al., (2003) in postmenopausal women with HRT (transdermal 17-estradiol (50 μg/day) and medroxyprogesterone acetate (10 mg/day) suggest that HRT itself may exert a beneficial effect on ocular symptomatology (ie blurring of vision due to dryness, feeling of dryness, tired eyes, etc) and increase lachrymal secretion. HRT treatment group also

resulting in reduce IOP and increase in corneal thickness (protective against development of POAG).

On the other hand, exposure to exogenous estrogen was a weakly protective factor against drusenoid deposit in AMD. Due to potential protective action by estrogen against the development of AMD, researchers investigated the effect of HRT in postmenopausal women. Haan MN et al., (2006) sought to determine whether HRT had a beneficial effect and whether different HRTs achieved different effects. They compared the efficacy of therapy based on conjugated equine estrogens (CEE) with CEE therapy combined with a progestinic. No association was found between the use of either therapy and the early development of AMD, suggesting that the early stages of AMD are not influenced by HRT. In contrast, combine therapy was more effective than CEE therapy alone in reducing the risk of developing both the drusenoid and neovascular forms of AMD. This is further supported by several other studies that has shown the exogenous estrogen plays a beneficial role in reducing the risk of advanced AMD (Feskanich et al., 2008 ; Edwards et al., 2010 ; Blasiak et al., 2014). Hence, female reproductive hormone may have protective effect against advanced AMD.

In a study by Nuzzi et al., (2018) that also support the role of estrogen on retina, they found that exposure to endogenous estrogens, depending on age at menarche and menopause and number of pregnancies, and exposure to exogenous estrogens, as in hormone replacement therapy and use of oral contraceptives, appear to protect against age related macular degeneration (both drusenoid and neurovascular types).

1.2.5 HRT and Its Effects on RNFL and Macular Thickness

Estrogen have the ability to influence the vascular tone and blood flow in organs and tissues (Tostes et al., 2003) and to have neuroprotective effects via nonvascular mechanisms (Green and Simpkins, 2000). In one study conducted by Desche et al., (2010) they found that HT (estrogens alone or in combination with progestogens) increases blood flow in a retinal artery and has a protective effect on the optic nerve head (ONH) and RNFL. They observed a greater blood flow in the inferotemporal retinal artery, mostly caused by its greater diameter, and a thicker neuroretinal rim and RNFL and these findings are in agreement with other clinical studies that used color Doppler imaging and reported improved pulsatility and/or resistivity indexes in retrobulbar vessels in women taking hormone replacement therapy.

In another cross sectional study by Na et al., 2014 regarding ocular benefits of estrogen replacement therapy, the prevalence of retinal nerve fiber layer (RNFL) defect was higher in the non-ERT group (OR = 1.703) compared to that in the ERT group.

A study conducted by Tharek et al., (2019) to investigate the effects of HRT in postmenopausal women on peripapillary RNFL, macular thickness and volume, and to compare them with postmenopausal women without HRT, they found that there is increased in macular thickness in superior outer macula (p=0.036) and positive correlation peripapillary RNFL at superotemporal area. This shows that HRT has promising effects on the retina particularly on macula.

1.2.6 Optical Coherence Tomography

OCT is based on the principle of low coherence interferometry, in which the signal carrying light returning from the eye is allowed to interfere with light that has travel a known path length. Infrared (830nm) incident beam created by a superluminescent diode source is divided into two beams by a beam splitter. One beam axially project to the patient's retina whilst the second beam (internal reference beam) is projected to a reference mirror at a known distance. When the two light beams (internal reference beam and the back scattered and back reflected light from retina) attempt to recombine at a detector, the reference beam must be altered in order to combine with the diagnostic beam. The amount the reference beam is altered compared to its baseline to match the probe signal (optical path length difference) results in signal generation (as illustrated in Fig 1.5)

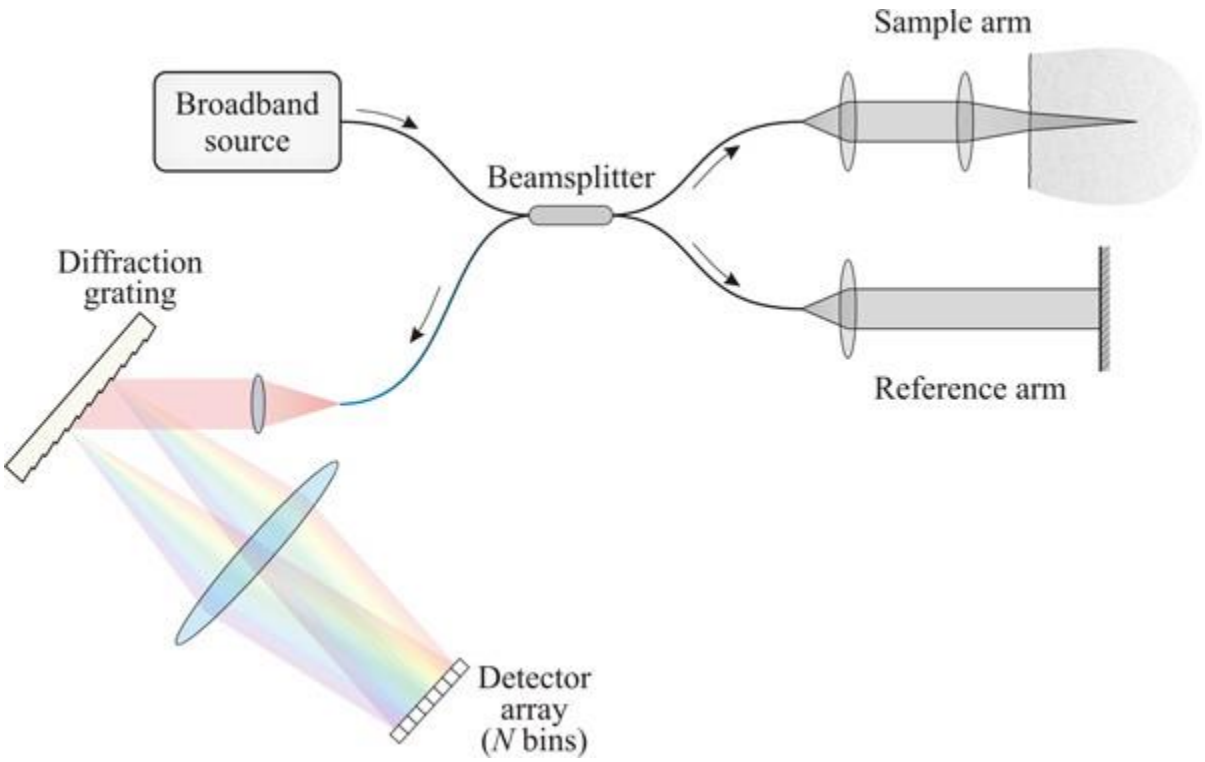


Figure 1.5 Schematic of SDOCT system

There are two main categories of OCT instrumentation: Time-Domain OCT (TDOCT) and Spectral-Domain OCT (SDOCT). Time-Domain OCT technology is more intuitive to understand, and most early research and commercial instrumentation was based on this technology. Spectral-Domain OCT is rapidly replacing the Time-Domain technology in most applications because it offers significant advantages in sensitivity and imaging speed.

While the earliest time-domain (TD) OCT technology could only acquire 400 scans per second, current SD-OCT models, depending on the manufacturer, can capture between 26,000 and 70,000 axial-scans per second. This improvement is beneficial to the clinician because it minimizes image artifacts, makes 3D imaging possible and increases image resolution.

Today's SD-OCT can also provide an axial resolution of 3µm to 6µm within tissues, compared with previous TD-OCT technology, which had a maximum resolution of 10µm. The increased speed and resolution provide an enhanced ability to visualize retinal layers.

OCT is indicated in diagnosis of anterior segment pathology, macular pathology and optic nerve pathology.

Clinicians have four prominent commercially available spectral-domain (SD) OCT models to choose from: Spectralis SD-OCT (Heidelberg Engineering), 3D OCT-2000 (Topcon Medical Systems), Avanti RTVue XR (Optovue) and Cirrus HD SD-OCT 5000 (Carl Zeiss Meditec).

In this study, Spectralis SD-OCT is used for obtaining RNFL and macula thickness as it is readily available in both, ophthalmology clinic HUSM and Hospital Kota Bharu. Features of

Spectralis SD-OCT machine includes scanning speed of 40,000 scans/s, scan depth is 1.9mm with axial resolution of 3.87µm and transverse resolution of 14µm (A Bayer, 2018).

The characteristics of good scan quality are good signal strength, no evidence of segmentation failure and blocked signal. Blocked signal can be caused by media opacity, pupil edge, drying of cornea, a smudge on the lens, posterior vitreous detachment and blinking. Of the scan quality related features, signal strength is the easiest to assess. A signal strength equal to or above 6 is desirable for the Cirrus HD-OCT (Carl Zeiss Meditec). For the Spectralis OCT (Heidelberg Engineering), the quality score should be greater than or equal to 20. For the Optovue, the signal strength index should be greater than or equal to 30. If the signal strength is lower, one may get artefactual thinning. Lower signal strength can be caused by media problems or the pupil size (Donald L.Budenz, 2016).

Signal strength variability was one of the leading factors associated with variability in measurement of RNFL thickness. Other factors include low analysis confidence (also called AC, a measure of quality of data as reported by the OCT software) and a low RNFL thickness. According to Z Wu et al., (2007) the comparability of RNFL thickness measurements between visits in known or suspected glaucomatous optic nerve changes may improve if the ophthalmologist could obtain scans of similar signal strength in each visit without a low AC. This is especially important for patients with moderate glaucomatous damage and for patients from whom good quality scans are not obtainable.

However, the sensitivity of imaging instruments in patients with moderate to advanced glaucoma -with low tissue thickness is not reliable. In this case, other test ie visual fields may be better in detecting progression.

1.2.7 Rationale of Study

Menopause is considered as a phase in a woman‘s life and one may wish that she can pass through this phase smoothly, but this may not be the case because of estrogen deficiency. HRT is the best treatment available for menopause not only for its positive effects systemically but ocular effects as well. Hence, this opportunity is used to study the effects of HRT on ocular structures.

Estrogen-replacement therapy prevents a decrease in end-diastolic velocity and an increase in ophthalmic arterial resistance index distal to the ophthalmic artery to levels matching those seen in young women (Harris-yitzhak et al., 2000). It also increases blood flow in a retinal artery specifically in the inferotemporal retinal artery, mostly caused by its greater diameter and has protective effect on ONH: a thicker neuroretinal rim and RNFL. Estrogens mediate arteriolar vasodilation through endothelial estrogen receptors, the activation of which is linked to local release of vasodilatory nitric oxide and prostaglandin I, and diminished release of vasoconstrictor endothelin -1(endothelium-derived contracting factors, which has been documented to be a potent vasoconstrictor of the ophthalmic and retinal arteries) (Deschene et al., 2010).

A few studies conducted show that postmenopausal women taking HRT has IOP significantly lower than in those who had never taken HRT (Sator MO et al., 1997; Uncu G et al., 2006; Tint NL et al., 2010). Hence, the HRT having protective effects against development of glaucoma. The mechanism is postulated due to improve in blood flow supplying ophthalmic and retinal artery causing protection of retinal ganglion cells and due to trabecular meshwork cells that contract in the presence of cholinergic agonists but relax with nitric oxide (NO) agonists, in the presence of estrogens, facilitating aqueous outflow.