THE EFFECT OF HONEY COCKTAIL SUPPLEMENT ON MACULAR THICKNESS, RETINAL NERVE FIBER LAYER THICKNESS AND OPTIC NERVE HEAD PARAMETERS IN POST-MENOPAUSAL WOMEN

BY

DR PREMALA DEVI SIVAGURUNATHAN

MD (UNIVERSITI MALAYSIA SABAH)

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF MEDICINE

(OPHTHALMOLOGY)

SCHOOL OF MEDICAL SCIENCES

UNIVERSITI SAINS MALAYSIA

2016

Disclaimer

I hereby clarify that the work in this dissertation is of my own except for quotations, some figures, and summaries which have been duly acknowledged.

……..

(Dr Premala Devi Sivagurunathan)

(PUM0020/12)

Date:

ACKNOWLEDMENTS

First and foremost, I am grateful to God for the good health and wellbeing that were necessary to complete this book.

I wish to express my sincere appreciation and gratitude to my supervisors for this masters program, Associate Professor Datin Dr Zunaina Embong, Consultant Ophthalmologist and senior lecturer at HUSM and Dr. Raja Norliza Raja Omar, Consultant Ophthalmologist at Hospital Melaka for their undivided guidance, attention to details and direction throughout this entire dissertation. Without their supervision, insightful comments and encouragement, this dissertation would not have been fruitful.

I also take this opportunity to express my appreciation to all lecturers in the Department of Ophthalmology, technicians, supporting staffs, and all my ophthalmology peers and colleagues for their constant support and ever willingness to help with the completion of this dissertation. In addition, I would like to extend my gratefulness to Dr Erika Kueh and Dr Ika from Department of Biostatistics for their time and contribution and guidance in preparing this dissertation.

Last but not least, I would also like to place on record my sense of indebtedness towards my family members for their undeterred support and encouragement. A special thank you to my beloved parents for all the support and sacrifices they have made for me in completing this study.

TABLE OF CONTENTS

DISCLAIMER ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS iv

LIST OF TABLES ix

LIST OF FIGURES xi

ABSTRAK xii

ABSTRACT xv

CHAPTER 1 INTRODUCTION 1

1.1 Introduction 1

1.2 Background 3

1.2.1 Menopause 3

1.2.2 Effects of Menopause 10

1.2.2.1 Systemic Effects 10

1.2.2.2 Ocular Effects 13

1.2.2.2.1 Macula Thickness 18

1.2.2.2.2 Retinal Nerve Fiber Layer Thickness 21

1.2.2.2.3 Optic Nerve Head Parameters 23

1.2.3 Treatment of Menopause 25

1.2.3.1 Hormone Replacement Therapy 25

1.2.3.2 Alternative Therapy 25

1.2.3.3 Honey Supplement 26

1.2.3.3.1 Content of Honey 26

1.2.3.3.2 Other Bee Products and Benefits 27

1.2.3.3.3 Honey Cocktail 28

1.2.3.3.4 Other Non-nutritional Components of Honey and Bee Products 29

1.2.3.3.5 Usage of Honey in Medical Conditions 30

1.2.3.3.6 Effects of Honey in Menopause 30

1.2.3.3.7 Effects of Honey in Ocular Tissue 31

1.2.4 Optical Coherence Tomography 33

1.2.5 Rationale of Study 34

CHAPTER 2 RESEARCH OBJECTIVES 35

2.1 General Objectives 35

2.2 Specific Objectives 35

CHAPTER 3 MATERIALS AND METHODS 36

3.1 Study Design 36

3.2 Place of Study 36

3.3 Study Duration 36

3.4 Study Population 36

3.5 Ethical Board Approval 36

3.6 Financial Support 37

3.7 Sample Size 37

3.8 Sampling Method 40

3.9 Randomization 40

3.10 Selection Criteria 40

3.10.1 Inclusion Criteria 40

3.10.2 Exclusion Criteria 40

3.11 Definition of Terms 41

3.11.1 Macular Thickness 41

3.11.2 Retinal Nerve Fiber Layer Thickness 43

3.11.3 Optic Nerve Head Parameters 44

3.11.4 Menopause 44

3.11.5 Honey 44

3.12 Instruments and Medications 45

3.12.1 Optical Coherence Tomography (OCT) (Cirrus HD-OCT 500, Carl Zeiss) 45

3.12.2 Honey Cocktail HC 124 46

3.12.3 Medications 47

3.12.3.1 Gutt Proparacaine 0.5 % (Alcon Laboratories, Texas, USA) 47

3.12.3.2 Gutt Tropicamide 1 % (Alcon Laboratories, Texas, USA) 47

3.12.3.3 Gutt Phenylephrine 2.5 % (Alcon Laboratories, Texas, USA) 47

3.13 Details of Methodology 48

3.13.1 Participants Recruitment 48

3.13.2 Baseline Parameters Measurements 50

3.13.2.1 Macular Thickness 50

3.13.2.2 Retinal Nerve Fiber Layer Thickness 50

3.13.2.3 Optic Nerve Head Parameters 51

3.13.3 Randomization 52

3.13.4 Honey Supplement 52

3.13.5 Follow Up 53

3.13.6 Evaluation at 3 months Post Supplement 54

3.14 Plans for Minimizing Errors 54

3.15 Statistical Analysis 54

CHAPTER 4 RESULTS 56

4.1 Demographic Data 56

4.2 Macular Thickness 58

4.2.1 Baseline of Mean Macular Thickness 58

4.2.2 Comparison of Mean Macular Thickness at Baseline and 3 Months in Post-Menopausal Women with Honey Cocktail Supplementation 60

4.2.3 Comparison of Mean Macular Thickness at Baseline and 3 Months in Post-Menopausal Women without Honey Cocktail Supplementation 62

4.2.4 Comparison of Mean Macular Thickness at 3 Months Post Supplement Therapy Between the Groups 64

4.2.5 Comparison of Mean Change in Macular Thickness Between the Groups 66

4.3 Retinal Nerve Fiber Layer Thickness 68

4.3.1 Baseline of Mean Retinal Nerve Fiber Layer Thickness 68

4.3.2 Comparison of Mean Retinal Nerve Fiber Layer Thickness at Baseline and 3 Months in Post-Menopausal Women with Honey Cocktail Supplementation 70

4.3.3 Comparison of Mean Retinal Nerve Fiber Layer Thickness at Baseline and 3 Months in Post-Menopausal Women without Honey Cocktail Supplementation 72

4.3.4 Comparison of Mean Retinal Nerve Fiber Layer Thickness at 3 Months Post Supplement Therapy Between the Groups 74

4.3.5 Comparison of Mean Change in Retinal Nerve Fiber Layer Thickness Between the Groups 76

4.4 Optic Nerve Head Parameters 78

4.4.1 Baseline Optic Nerve Head Parameters 78

4.4.2 Comparison of Optic Nerve Head Parameters at Baseline and 3 Months in Post-Menopausal Women with Honey Cocktail Supplementation 79

4.4.3 Comparison of Optic Nerve Head Parameters at Baseline and 3 Months in Post-Menopausal Women without Honey Cocktail Supplementation 80

4.4.4 Comparison of Optic Nerve Head Parameters at 3 Months Post Supplement Therapy Between the Groups 81

4.4.5 Comparison of Mean Change in Optic Nerve Head Parameters Between the Groups 82

CHAPTER 5 DISCUSSION 83

5.1 Demographic and Reproductive Factors Characteristics 83

5.2 Effect of Honey Cocktail on Macular Thickness, Retinal Nerve Fiber Layer Thickness andOptic Nerve Head Parameters in Post-Menopausal Women 85

5.3 Limitations and Recommendations 97

CHAPTER 6 CONCLUSION 99

CHAPTER 7 REFERENCES 100

CHAPTER 8 APPENDICES 113

Appendix A-Ethical Approval USM 113

Appendix B-Information Sheet 114

Appendix C-Consent Forms 121

Appendix D-Data Report Form 123

Appendix E-Flow Chart 124

\\

LIST OF TABLES

Table 1.1 Hormonal values of the hypothalamic-pituitary-gonadal axis by menopause stage 5

Table 4.1 Demographic data for both groups 57

Table 4.2 Baseline of mean macular thickness for both groups 59

Table 4.3 Comparison of mean macular thickness at baseline and 3 months in post-menopausal women with honey cocktail supplementation 61

Table 4.4 Comparison of mean macular thickness at baseline and 3 months in post-menopausal women without honey cocktail supplementation 63

Table 4.5 Comparison of mean macular thickness at 3 months post supplement therapy between the groups 65

Table 4.6 Comparison of mean change in macular thickness between the groups 67

Table 4.7 Baseline of mean retinal nerve fiber layer thickness for both groups 69

Table 4.8 Comparison of mean retinal nerve fiber layer thickness at baseline and 3 months in post-menopausal women with honey cocktail supplementation 71

Table 4.9 Comparison of mean retinal nerve fiber layer thickness at baseline and 3 months in post-menopausal women without honey cocktail supplementation 73

Table 4.10 Comparison of mean retinal nerve fiber layer thickness at 3 months post supplement therapy between the groups 75

Table 4.11 Comparison of mean change in retinal nerve fiber layer thickness between the groups 77

Table 4.12 Baseline of optic nerve head parameters for both groups 78

Table 4.13 Comparison of optic nerve head parameters at baseline and 3 months in post-menopausal women with honey cocktail supplementation 79

Table 4.14 Comparison of optic nerve head parameters at baseline and 3 months in post-menopausal women without honey cocktail supplementation 80

Table 4.15 Comparison of mean optic nerve head parameters at 3 months post supplement therapy between the groups 81

Table 4.16 Comparison of mean change in optic nerve head parameters between the groups 82

LIST OF FIGURES

Figure 1.1 Transition period from pre-menopause to post-menopause in relation to estrogen level (Adapted from Pauwels *et al*., 2008) 6

Figure 1.2 Chemical structure of estradiol, estriol and estrone (Adapted from Pauwels *et al.*, 2008) 7

Figure 1.3 Hormonal level throughout a woman's life (A), hormonal fluctuations during menopause (B) and female testosterone decline with age (C) (Adapted from Deuter *et al*., 2014-2015) 9

Figure 1.4 Distribution of estrogen receptor in the retinal layers (Adapted from Munnaut *et al*., 2001) 16

Figure 1.5 Distribution of estrogen receptor mRNA in the centre and periphery of retina (Adapted from Munnaut *et al*., 2001) 16

Figure 3.1 Macular thickness mapping involving 9 areas as described by ETDRS study (ETDRS, 1985) 42

Figure 3.2 Macular thickness mapping taken using Cirrus HD-OCT 42

Figure 3.3 Retinal nerve fiber layer mapping involving 4 quadrants 43

Figure 3.4 Retinal nerve fiber layer and optic nerve head mapping by Cirrus HD-OCT 43

Figure 3.5 Cirrus HD-OCT Spectral Domain Technology Carl Zeiss, Germany 45

Figure 3.6 Honey Cocktail HC 124 46

Figure 3.7 Topical proparacaine 0.5 %, topical tropicamide 1 %, topical phenylephrine 2.5 % (from left to right) 48

Figure 5.1 Chemical structure of estrogen (A) compared to flavonoids chemical structures in honey (B) (Adapted from Ogueta *et al.,* 1999) 88

Figure 5.2 Distribution of blood vessels in the retinal layers (Adapted from *Hood et al*.,2008) 93

ABSTRAK

Pengenalan

Menopaus merupakan satu fasa yang penting dalam kehidupan wanita. Perubahan klinikal yang berlaku pada peringkat umur ini bukan sahaja melibatkan struktur *vasomotor*, *genitourinari*, gangguan tidur, dan perubahan keadaan jiwa, tetapi juga melibatkan struktur mata misalnya lapisan air mata, kanta mata, tekanan mata, pengaliran darah dalam mata dan juga retina. Selain daripada terapi hormone (HRT), terdapat terapi alternative termasuk pengamalan madu yang menunjukkan kesan yang baik dalam merawat simptom menopaus and juga dalam merawat penyakit mata dan mengekalkan fungsi struktur mata.

Objektif

Matlamat kajian ini adalah untuk membandingkan ketebalan makula, ketebalan urat saraf retina dan parameter kepala saraf optik di kalangan wanita menopaus yang mengamalkan pengambilan produk madu koktail

Kaedah Kajian

Kajian ini merupakan kajian prospektif terkawal secara rawak yang dikendalikan di Hospital Universiti Sains Malaysia antara bulan Mac 2014 dan Julai 2015. Seramai 60 wanita menopaus yang memenuhi kriteria pemilihan telah dipilih dan di rawakkan ke dalam 2 kumpulan menggunakan teknik rawak sampul surat legap, kumpulan “MADU KOKTAIL” dan kumpulan “TANPA MADU”. Paras ketebalan makula dasar, ketebalan urat saraf retina dasar dan parameter

kepala saraf optik dasar diambil menggunakan mesin OCT Cirrus HD oleh pegawai terlatih tanpa mengetahui kumpulan kajian. Peserta dalam kumpulan “MADU KOKTAIL” diarahkan untuk mengambil madu koktail yang dibekalkan untuk tempoh 3 bulan. Peserta perlu mengambil sebungkus madu koktail yang mengandungi 20 gram satu hari terus daripada bungkusan tanpa melarutkannya. Peserta dalam kumpulan “TANPA MADU” tidak dibekalkan *placebo*. Selepas 3 bulan, semua paras ketebalan makula, ketebalan urat saraf retina and parameter kepala saraf optic diulangi menggunakan mesin, pegawai and teknik yang sama. Analisa statistik dijalankan mengggunakan SPSS versi 22.0

Keputusan

Seramai 60 peserta telah dipilih (30 peserta dalam setiap kumpulan iaitu kumpulan madu koktail and kumpulan tanpa madu. Purata keseluruhan ketebalan makula menunjukkan ketebalan yang ketara dengan pengambilan madu koktail di kalangan wanita menopaus 3 bulan selepas rawatan ($p = 0.002$).Terdapat juga perbezaan yang ketara dalam perubahan purata keseluruhan dalam ketebalan makula pada 3 bulan selepas rawatan di antara kedua-dua kumpulan ($p <0.001$). Purata keseluruhan ketebalan urat saraf retina menunjukkan ketebalan yang ketara dengan pengambilan madu koktail di kalangan wanita menopaus 3 bulan selepas intervensi ($p = 0.033$). Terdapat juga perbezaan yang ketara dalam perubahan purata keseluruhan dalam ketebalan urat saraf retina pada 3 bulan selepas rawatan di antara kedua-dua kumpulan ($p < 0.001$). Tiada perbezaan yang ketara dalam parameter paras kepala saraf optik di kalangan wanita menopaus antara kumpulam madu koktail dengan dan tanpa pengambilan madu 3 bulan selepas rawatan. Terdapat perbezaan yang ketara dalam perubahan purata kawasan lingkaran ($p = 0.003$), kawasan cawan ($p = 0.001$)

dan nisbah cawan kepada cakera ($p < 0.001$) pada masa 3 bulan selepas rawatan di antara kedua-dua kumpulan.

Kesimpulan

Madu memaparkan kesan positif dalam melindungi ketebalan makula, urat saraf retina dan juga parameter kepala saraf optik di kalangan wanita menopaus.

ABSTRACT

Introduction

Menopause is an integral part of a woman's life. The clinical changes that occur during this period of life not only involve vasomotor, genitourinary, sleep disturbances and mood changes but also involve ocular structures such as tear film, lens, intraocular pressure, ocular blood flow and the retina. Apart from hormone replacement therapy (HRT), many other alternative therapies including honey showed promising effect in relieving menopausal symptoms, treating ocular diseases and maintain ocular functions.

Objective

To compare mean macular thickness, retinal nerve fiber layer (RNFL) thickness and optic nerve head (ONH) parameters with and without honey cocktail supplement in post-menopausal women.

Methods

This is a prospective randomized controlled trial conducted in Hospital Universiti Sains Malaysia between March 2014 and July 2015. A total of 60 post-menopausal women who fulfilled the selection criterias were selected and randomized into 2 groups using randomized opaque envelope technique "HONEY COCKTAIL" and "NO HONEY". Baseline macular thickness, RNFL thickness and ONH parameters were taken using Cirrus HD-OCT machine for both groups by blinded trained personnel. Participants in the "HONEY COCKTAIL" group were

instructed to take honey cocktail from the honey cocktail sachets provided for a total of 3 months duration. The dosing was 1 sachet containing 20 grams per day consumed straight from the sachet without dilution. Participant in the "NO HONEY" group were not given any placebo. After 3 months, the macular thickness, RNFL thickness and ONH parameters were repeated using the same machine, same technique and by the same blinded medical personnel. Statistical analysis was performed using SPSS version 22.0.

Results

A total of 60 participants were recruited (30 participants in each honey cocktail and no honey group). The mean global macular thickness was significantly thicker in post-menopausal women with honey supplement 3 months post supplement therapy ($p = 0.002$). There was also a significant difference in the mean change of global macular thickness at 3 months post supplement therapy between the 2 groups ($p < 0.001$). The mean global RNFL thickness was significantly higher in post-menopausal women with honey supplement 3 months post intervention ($p = 0.033$). There was also a significant difference in the mean change of global RNFL thickness at 3 months post supplement therapy between the 2 groups ($p < 0.001$). There were no significant difference in the ONH parameters in post-menopausal women with and without honey cocktail supplement 3 months post supplement therapy. There was significant difference in the mean change of rim area ($p = 0.003$), cup area ($p = 0.001$) and cup-disc-ratio ($p <0.001$) at 3 months post supplement therapy between the 2 groups.

Conclusion

Honey cocktail was shown to be beneficial and protective in improving macular thickness, RNFL thickness and ONH parameters in post-menopausal women.

CHAPTER 1

INTRODUCTION

1.1 Introduction

One of the most important and integral part of a woman's life is menopause. This typically happens after 12 months period of amenorrhea that occurs after the final menstrual period. The clinical manifestation of menopause not only involves systems such as vasomotor, genitourinary, sleep disturbances and mood changes but also involves the ocular structures such as tear film, lens, intraocular pressure, ocular blood flow and retina (Altintas *et al.*, 2004).

Hormone replacement therapy (HRT) has been the mainstay of treatment for decades to aid women in coping with changes in their post-menopausal time frame. This therapy has been found to be beneficial in protecting ocular structural changes related to menopause as well. In a study done among Korean post-menopausal women, it was found that estrogen replacement therapy was protective against ocular conditions such as the development of cataract, pterygium, and retinal nerve fiber layer defects (Na *et al.*, 2014).

However, in recent years, there's a paradigm shift towards alternative therapy to help women cope with the unpleasant symptoms of menopause and yet lead a normal life. This is due to the concerns that HRT could increase the risk of ischemic stroke, cardiovascular diseases and breast cancer (Sulaiha *et al.*, 2010). Some of these alternative therapies which are of interest include acupuncture, herbal remedies such as evening prime rose oil, ginseng, black cohosh, red clover and soy supplement and gingko biloba extract(Geller and Studee, 2005). However, studies done

on these remedies are very limited and the results are inconsistent. Besides this, the effects of these remedies on ocular structures are not known and require further elaborative studies.

Lately, there's an interest in using honey as a modality of treatment for conditions related to menopause and several studies have showed promising results (Zaid *et al.*, 2012; Zaid *et al.*, 2010a). Through these studies, we have learned that honey has a positive effect on estrogen sensitive tissues. Ocular structures such as the cornea and retina has been proven to have estrogen receptors (Ogueta *et al.*, 1999; Tachibana *et al.*, 2000). Therefore, it can be postulated that honey supplement may have an estrogenic effect on the retina and improve retinal parameters in post-menopausal women. In addition, honey cocktail which comprise of bee pollen and royal jelly apart from honey has been shown to have similar estrogenic effect as honey. Hence, it is postulated that the effect on retinal parameters such as macular thickness, retinal nerve fiber layer thickness will be similar as honey and when combined together the effect will much more enhanced.

In addition, other studies have also demonstrated honey to be a good antioxidant (Bashkaran *et al.*, 2011), anti-inflammatory (Bashkaran *et al.*, 2011) and a good rheological agent (Khalil M.I, 2010). Thus, it is also postulated that these beneficial properties of honey play important role in the retina and can improve retinal parameters in post-menopausal women. Moreover, honey is a natural substance that readily available, easy to consume and with minimal or no side effects.

1.1 Background

1.2.1 Menopause

Menopause is a natural progression in a women's life and in women's physiology. It is a stage in life when a woman stops having her monthly menstruation. It is an integral part of aging and marks the end of a woman's reproductive years. Menopause typically occurs in a woman's late 40s to early 50s. In Malaysia, the average life expectancy in women ranges from 68 years to 75.2 years and about one third of women's life is spent in menopause. In a study conducted in Singapore found that the mean age of menopause is 49.0 years. This was not significantly different between the three ethnic groups (Chinese, Malay and Indians) (Loh *et al.*, 2005). In another study looking at the prevalence of menopause in Kelantan, it was found that the mean age at menopause was 49.4+/-3.4(SD) (Dhillon *et al.*, 2006).

Natural menopause is the permanent ending of menstruation that is not brought on by any type of medical treatment whereas surgical menopause refers to menopause attained in an ovariectomized woman. The term climacteric was first coined by Stoppard in 1994 and is defined as a phase of transition in a woman's life whereby their ovarian function and hormonal production begin to decline. It can be further divided into pre-menopause, peri-menopause, menopause and post-menopause (Stoppard, 1994).

The term pre-menopause refers to the entirety of a woman's life from the first to her last regular menstrual period (Stoppard, 1994). Therefore, pre-menopause is best defined as a period of time of "normal" reproductive function in a woman leading into the stage of menopause called peri-menopause.

According to World Health Organization (WHO), peri-menopause is a period of 2-8 year time frame preceeding menopause and before complete cessation of the menstrual period. Peri-menopause usually appears in women from 35-50 years of age. This stage of menopause is characterized by hormone fluctuations, which cause the typical menopause like menstrual irregularities. The hormonal balance between estrogen, progesterone, follicle stimulating hormone (FSH) and lutenizing hormone (LH) in the normal menstrual cycle is altered and these hormonal fluctuation results in the symptoms of peri-menopause. During this period of time also, FSH and LH levels will start to rise and will be higher. The ovaries at this time will produce less estrogen and therefore the estrogen level tends to be lower (Rizk DEE, 1998). The hormonal levels of the hypothalamic-pituitary-gonadal axis at peri-menopause stage is shown in Table 1.1.

Table 1.1 Hormonal values of the hypothalamic-pituitary-gonadal axis by menopause stage (Navarro *et al.*, 2012)

Hormone	Eumenorrheic (n = 42) X±DE	Perimenopausal (n = 55) X±DE	Postmenopausal		p Value for trend
			Early stage (n = 78) X±DE	Late stage (n = 55) X±DE	
FSH IU/L	6.97±3.8	34.69±11.24	75.43±26.3	73.08±56	0.003
LH IU/L	4.23±1.123	20.78±10.33	37.59±19.33	32.44±18.3	<0.001
Estradiol pmol/L	314±150	201±45	117±40	80.26±17.2	0.379
Testosterone nmol/L	1.022±0.23	0.835±0.012	0.847±0.05	0.959±0.18	0.725

During the last 1-2 years of peri-menopause, the drop in estrogen accelerates and peri-menopause prolongs until to the point when the ovaries stop releasing eggs. Women are still having menstrual cycles during peri-menopause, and can get pregnant.

Menopause represents the end stage of a natural transition in a woman's reproductive life. Menopause is the point at which estrogen and progesterone production decreases permanently to very low levels. In menopause the ovaries stop producing eggs, there's a permanent cessation of a woman's menstrual cycle and a woman is no longer able to get pregnant naturally.

Post-menopause on the other hand, is a span of life time after the last menstrual period (Stoppard, 1994). Post-menopausal period is 12 consecutive months of amenorrhea after the last

menstruation. In post-menopause a woman's hormone patterns have changed significantly, because the ovaries are no longer producing estrogen or progesterone. Instead of them fat cells continue to produce estrogen at around 40 per cent of previous levels. The ovaries have now begun to shrink in size but still have quite an important role in post-menopause since some hormones continue to be produced there. The transition period from pre-menopause to post-menopause is shown in Figure 1.1.

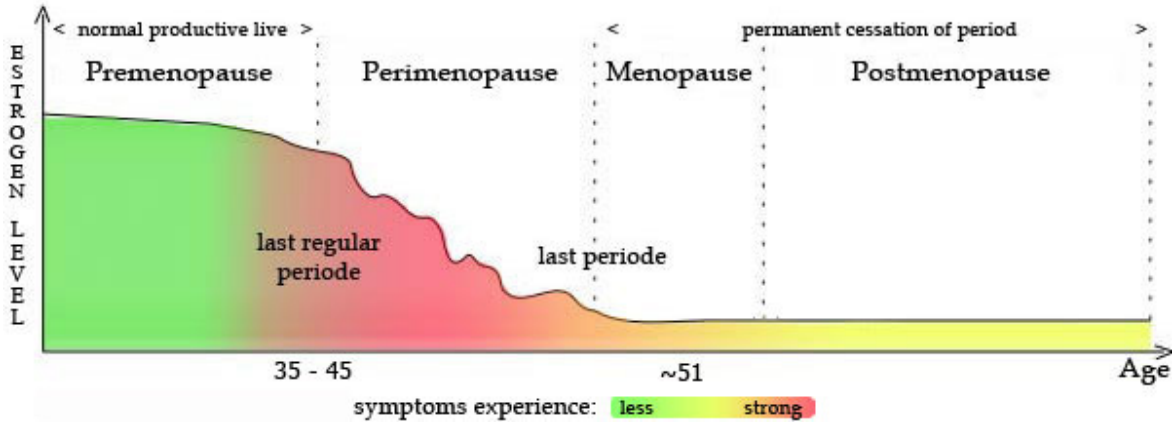


Figure 1.1 Transition period from pre-menopause to post-menopause in relation to estrogen level (Adapted from Pauwels *et al*., 2008)

As studies have shown, in post-menopausal women, there's a drastic change in the hormonal status in comparison with normal menstrual cycle. Among all the hormones in the female reproductive cycle, estrogen, being a major steroidal female sex hormone produced by the ovarian follicles plays a major role and is responsible for the female menstrual cycle and

reproductive cycle. It can be classified into three types which include estriol, estradiol and estrone. The chemical structures of these hormones are shown in Figure 1.2.

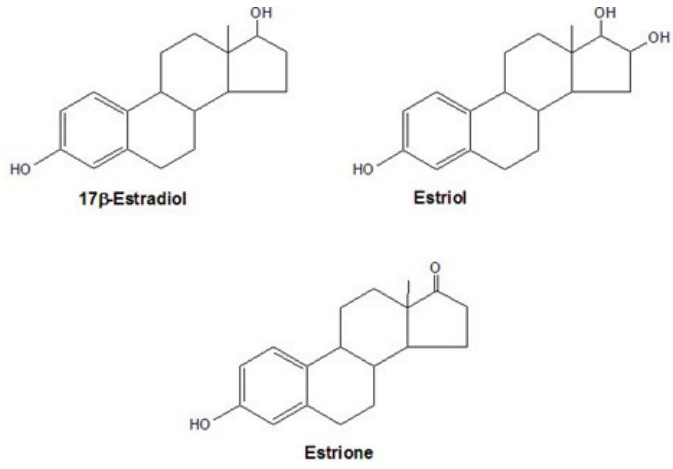
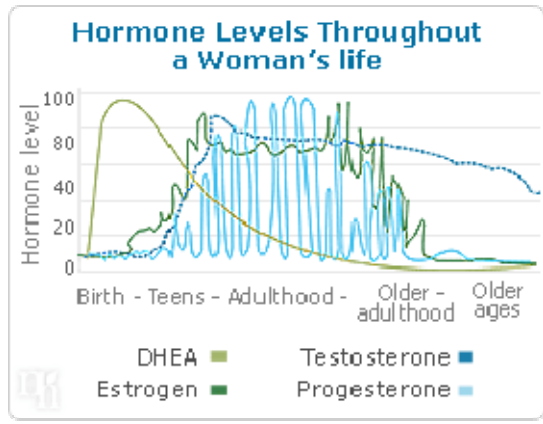


Figure 1.2 Chemical structure of estradiol, estriol and estrone (Adapted from (Pauwels *et al.*, 2008)

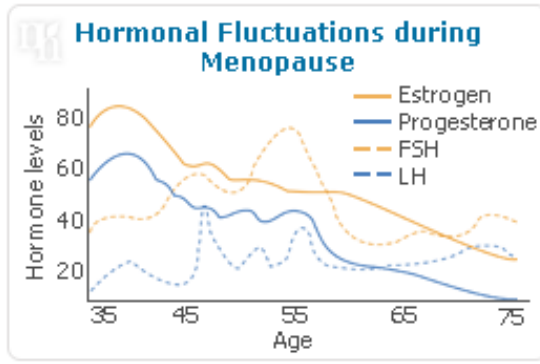
Among the three subtypes, estradiol is the "active" estrogen produced by the ovaries and is the most potent. Estrone on the other hand is considered a weaker form of estrogen. It is typically produced by special belly fat cells, and is the major estrogenic form found in naturally-menopausal women who are not taking HRT. Estriol is a metabolic waste product of estradiol metabolism that can still have some effects upon a limited number of estrogen receptors. It is 8% as potent as estradiol and 14% as potent as estrone. Once estriol is bound to an estrogen receptor, it blocks the stronger estradiol from acting there. Thus it is considered to have both estrogenic and antiestrogenic actions (Secky *et al.*, 2013). Estradiol is the primary sex hormone of childbearing women and is responsible for female characteristic and sexual functioning. The main function of estradiol is stimulating growth of breast tissue, maintenance of vaginal blood

flow and lubrication, causes lining of the uterus to thicken during the menstrual cycle, keeps vaginal lining elastic, and preserving bone density. Other important female hormone includes progesterone which aids in the preparation of the lining of the uterus for a fertilized egg and helps maintain early pregnancy. Although known as the "male" hormone, testosterone is also found in women and is also important to women's sexual health as it plays a key role in women's estrogen production, contributes to libido and helps to maintain bone and muscle mass.

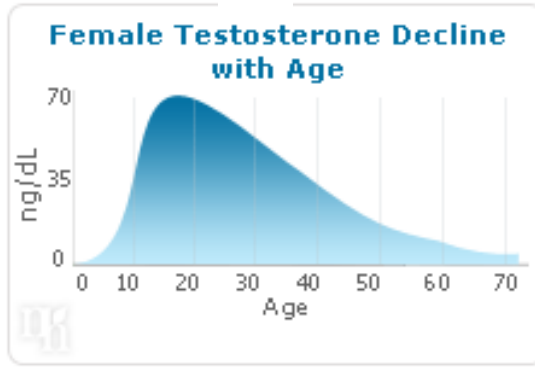
During menopause, estrogen levels fluctuate and become unpredictable. Eventually, production falls to a very low level. In post-menopausal women, estradiol and estrone decline from 62.5% to 4% of the pre-menopausal level (Vermeulan, 1976). Progesterone production stops during menstrual cycles when there is no ovulation and after final menstrual period. Testosterone levels on the other hand peak in a woman's 20s and decline slowly thereafter. By menopause, testosterone level is at half of its peak. Ovaries continue to make testosterone even after estrogen production stops and testosterone production from adrenal glands also declines with aging but continues after menopause. Studies showed a 50% reduction of androgen in menopausal women with intact ovaries (Genazzani *et al.,* 1999). The hormonal changes that occur during a women's lifetime and menopause is shown in Figure 1.3A, 1.3B and 1.3C.



A



B



C

Figure 1.3 Hormonal level throughout a woman's life (A), hormonal fluctuations during menopause (B) and female testosterone decline with age (C) (Adapted from Deuter *et al*., 2014-2015)

1.2.2 Effects of Menopause

1.2.2.1 Systemic Effects

During the transitional period of menopause, women may experience vasomotor symptoms such as hot flushes, night sweats and or cold flashes, insomnia, emotional changes such as irritability, mood swings, and mild depression. Apart from this, patients also experience uro-genital symptoms such as urinary frequency, vaginal dryness and dyspareunia. In a study conducted in Kelantan looking at prevalence of menopausal symptoms in women found that 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. Some patients had atypical symptoms and the prevalence of atypical symptoms was as follows: tiredness (79.1%), reduced level of concentration (77.5%), musculo-skeletal aches (70.6%) and backache (67.7%) (Dhillon *et al.*, 2006).

The long term health risks associated with menopause include osteoporosis, coronary artery disease and decline in cognitive function. Estrogen plays an important role in preserving bone mass. Estradiol especially acts directly on osteoclast by reducing the rate of bone resorption (Steinweg, 2002). Many studies done showed that post-menopausal women had lower bone mineral density and the decrease in estradiol directly contributes to this. Generally, women lose an average of 25% of their bone mass from the time of menopause to age 60. In a study looking

at factors affecting bone loss in menopausal women without hormone replacement therapy, it was found that, in women under 5 years post-menopausal at baseline, bone loss rate was significantly lower than in peri-menopausal women. In women over 5 years post-menopausal at baseline, bone loss rate was significantly further decreased only at lumbar spine (Sirola *et al.*, 2003). Over time, this loss of bone can lead to bone fractures. In addition to this, post-menopausal state is also associated with high incidence of cardiovascular disease. This is due to the emergence of metabolic syndromes which include increase in central and intra-abdominal body fat, a change toward more atherogenic lipid profile, with increased low density lipoprotein particles and increased insulin resistance (Knopp, 2002).

In other studies looking at the correlation between osteoporosis, arterial stiffness and atherosclerosis, it was found that women with osteoporosis had significantly higher arterial stiffness and higher arterial stiffness was further associated with the presence of atherosclerosis and this provided initial data suggesting that women with osteoporosis may have a higher risk of developing coronary atherosclerosis (Seo *et al.*, 2009).

Apart from this, it is also suggested that decline in estrogen around menopause are associated with deterioration in cognitive function as well as increased risk of depressive symptoms and depressive disorder (Weber *et al.*, 2014). Cognitive function comprises various components but the most important aspect that is affected by estrogen decline is verbal learning and memory. Clinically, the protective effects of estrogen specifically target the cognitive domains that are the most vulnerable to aging in both female rats (Markowska and Savonenko, 2002) and women

(Sherwin, 1988). This is due to a decline in estrogen which is largely concentrated at hypothalamus, amygdala and hippocampus in menopause (Shughrue and Merchenthaler, 2000). Post-menopausal women performed significantly worse than pre- and peri-menopausal women on delayed verbal memory tasks, and significantly worse than peri-menopausal women on phonemic verbal fluency tasks. Peri-and post-menopausal women were at significantly increased risk of depression, as measured by standard symptom inventories and structured clinical interviews, than pre-menopausal women (Weber *et al.*, 2014). Therefore, menopausal transition is a time of increased vulnerability to cognitive deterioration and increased risk of depressive symptoms and depressive disorders.

1.2.2.2 Ocular Effects

The incidence of eye manifestations not only increases with age but there exist a gender base difference as well. This is most likely due to the presence of sex steroid hormone receptors in the ocular tissues. There's a strong correlation between estrogen and changes in ocular tissue and these changes are more prevalent in the post-menopausal period due to the decline in estrogen.

Dry eye is more common in females and studies have established that androgen controls the various biochemical and physiological aspects of the lacrimal apparatus and this hormone is generally deficient in females (Sharma and Hindman, 2014). Since women have lower levels of androgens compared to the levels in men and further age-related decreases in androgen levels may diminish the androgen levels below the critical amount needed for optimum eye health. Along with lower androgen levels, post-menopausal women develop lower levels of estrogen that is known to stimulate meibomian glands and help regulate ocular surface homeostasis. Therefore, a combination of androgen and estrogen deficiency lead to inadequate lacrimal gland secretion with superimposed tear-film instability in older women and higher risk of developing dry eye (Sriprasert *et al.*, 2015). This observations have been further strengthened by the increased association of dry eye during pregnancy and lactation (Sullivan, 2004). Apart from this, the conjunctival epithelium seems to show cyclic variations during menstruation and menopause. Even the maturity of the conjunctival tissue strongly correlates with the levels of estrogen (Wagner *et al.*, 2008).

Besides this, the incidence of cataract is much higher in post-menopausal females as compared to males of similar age groups and it appears that sex hormones have a significant role in prevention of cataract formation in post-menopausal women as observed by one large study. The protective action of estrogen is most likely to maintain the ionic composition and hydration status in the lens and thus preventing cataract formation (Gupta *et al.*, 2005; Leske *et al.*, 2004).

Post-menopausal women have lower contrast sensitivity detection and elevated intraocular pressure (IOP) compared to pre-menopausal women (Siesky *et al.*, 2008). In another study conducted in South India found that post-menopausal women had higher IOP that could be ascribed to alternating levels of oestrogen and progesterone after menopause, which could act by altering any/several components of the IOP regulating mechanisms (Panchami *et al.*, 2013). Central corneal thickness also was significantly decreased in post-menopausal women compared to pre-menopausal women (Keskin *et al.*, 2009).

There's also evidence to suggest a relationship between estrogen and retinal disorders. Through different known and unknown mechanisms, estrogen exerts a protective role in preventing retinal changes by various genomic and non-genomic effects (Evans *et al.*, 1998). This estrogenic effect on the retina is possible due to the presence of estrogen receptors in the retina.

In recent years, studies done in rats, bovine and human have demonstrated the presence of estrogen receptor in the retina (Kobayashi *et al.*, 1998; L. Alexandra Wickham, 2000; Munaut *et*

al., 2001; Ogueta *et al.*, 1999). Based on the study done by Munaut *et al*. (2001), 2 types of estrogen receptor were identified in the human retina which were estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). Analysis using immune chemical localisation, western blot and rapid time polymerase chain reaction (RT-PCR) were done in neural retina, retinal pigment epithelium and choroid layer to demonstrate the presence of these receptors. The study demonstrated the presence of ERα and ERβ in the neural retina and retinal pigment epithelium (RPE)-choroid complex. Expression of ERβ was more constant and evenly distributed in the retina, mainly observed in the ganglion cell layer and choroid while expression of ERα was unequally distributed between the retina, especially the ganglion cell layer and the RPE-choroid complex. Distribution of estrogen receptor in the retinal layers and the distribution of estrogen receptor messenger ribonucleic acid (mRNA) in the centre and periphery of the retina is shown in Figure 5.1 and 5.2 accordingly.

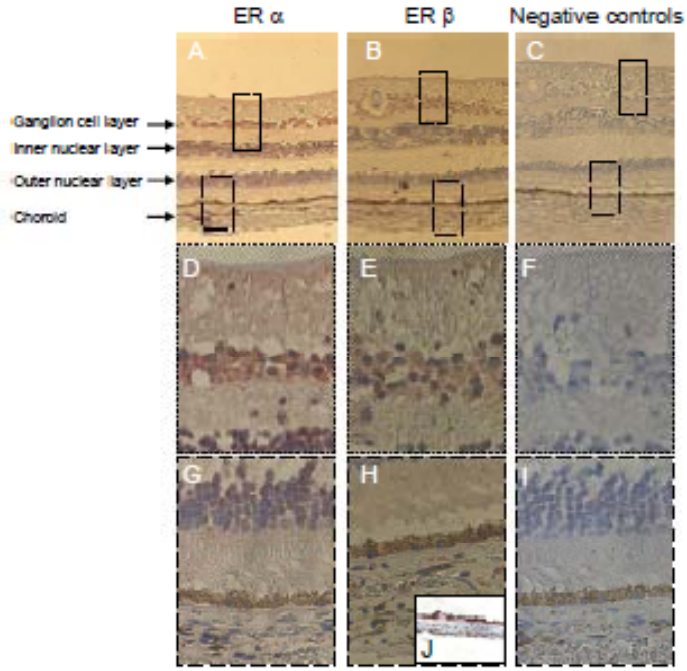


Figure 1.4 Distribution of estrogen receptor in the retinal layers

(Adapted from Munnaut *et al*., 2001)

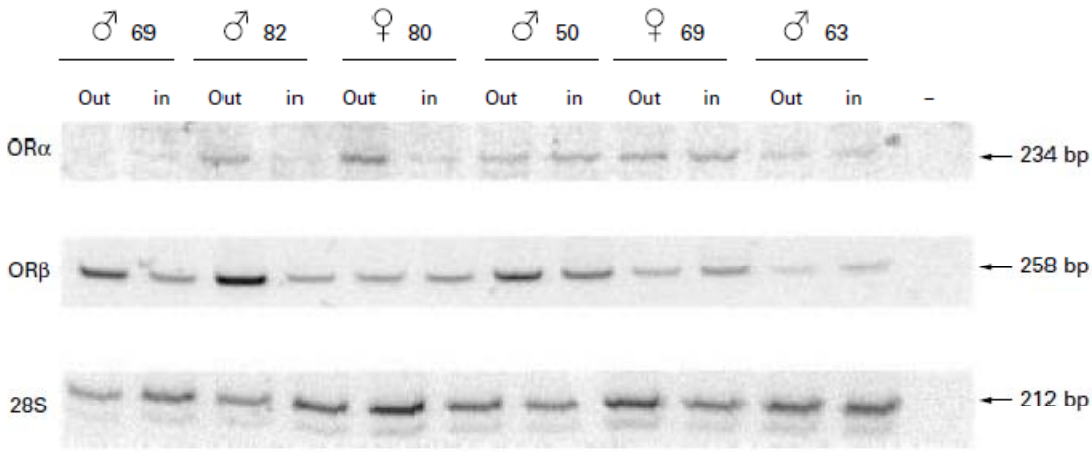


Figure 1.5 Distribution of estrogen receptor mRNA in the centre and the periphery of the retina

(Adapted from Munnaut *et* al., 2001)

In another study done to assess the influence of estrus cycle on the retinal structure and function between aging male and female rats, it was discovered that the electro-retinography (ERG) amplitudes were larger in pre-menopausal rats compared to menopausal female rats and were also larger than those obtained from age-matched male rats. In contrast, following menopause, the ERG responses in female rats declined dramatically compared to age-matched male rats. These findings suggest a beneficial effect of the estrus cycle and its related fluctuating hormonal changes, especially estrogen upon the retinal function. This may further explain the higher prevalence of age-related retinal degeneration in women after menopause (Chaychi *et al.*, 2015). Increased incidence of macular degeneration in post-menopausal women due to deficiency of estrogen was also observed by the Eye Disease Case–Control Study Group (Evans *et al.*, 1998).

1.2.2.2.1 Macular Thickness

Macular is a very important structure in the human eye and is the most sensitive part of the retina. Disease which affects the macula causes visual disruption and affects the quality of life. The most common form of macular disease is age related macular degeneration (AMD). It is the leading cause of visual loss and blindness among older adults. The prevalence of AMD in the United States is 1.75 million and is expected to increase to 3 million by 2020 (Mangione *et al.*, 1999). This disease is associated with many factors such as aging, smoking, and genetics and is more common among female patients especially after the age of 65 and the incidence increases with age in females. In AMD there is deposition of lipofuscin at the Bruch membrane, further causing thinning and atrophy of retinal pigment epithelial cells.

The researchers suggested that oxidative stress plays an important role in the degeneration of the retinal epithelium. In women, the antioxidant properties 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, the antioxidant level is low due to decrease in estrogen and other related factors and the reduction of antioxidant increases the oxidative stress on the retina and therefore makes these women more prone to AMD.

Based on several studies, endogenous estrogen exposure is associated with the risk of AMD. The Aravind Comprehensive Eye Survey showed age at menarche ≥14 years, is a risk factor for overall AMD (OR, 2.3) (Nirmalan *et al.*, 2004), and the Rotterdam Study showed an increased risk of AMD in those who experienced early menopause following oophorectomy (OR, 3.8) (Tomany *et al.*, 2004). The Blue Mountain Eye Study reported that a long reproductive period was associated with a decreased prevalence of early AMD (Smith *et al.*, 1997). These findings provide further support for the notion that a shorter duration of estrogen exposure may increase the risk of AMD. Apart from this, researchers have also shown that menopausal females not on HRT have a higher risk to develop AMD compared to menopausal females on HRT (Klein, 2000). Hence, female reproductive hormone especially estrogen may have protective effect against AMD.

Apart from AMD, other macular diseases such as idiopathic macular hole which accounts for 0.3% of macular disease has been shown to have a higher prevalence in females with female: male ratio of 3:1 (Morgan and Schatz, 1986). However the underlying cause for this difference is unknown.

Several studies done showed that there is a difference in macular thickness between males and females (Adhi *et al.*, 2012; Murthy *et al.*, 2015; Song *et al.*, 2010; Wexler *et al.*, 2010; Wong *et al.*, 2005). This thickness appears to be more in males. The difference in macular thickness varies with age and is more distinct in older men and women. Only a few studies did not find gender differences in macular thickness (Chan *et al.*, 2006; Grover *et al.*, 2009; Sull *et al.*, 2010).

Therefore based on these findings, it is postulate that the disparity of macular thickness between males and females can be due to influence of sex hormones.

So far, there is very limited data on the normative data of macular thickness in post-menopausal women compared to reproductive age group women to assess whether there is an effect of female reproductive hormone on macula thickness. Based on a population based study carried out by Atas *et al*. (2014) in evaluating macular thickness, RNFL and choroid thickness in post-menopausal women and reproductive age group, they found that after adjusting for age there was significant thinning of the central subfield, and temporal inner macula in post-menopausal women compared to reproductive age group women. This shows that female reproductive hormone do influence the macula thickness.

1.2.2.2.2 Retinal Nerve Fiber Layer Thickness

Retinal nerve fiber layer (RNFL) is the innermost layer of the retina which consists of axons and ganglion cells that follow a characteristic pattern towards the optic disc. The fibers exit the eye as optic nerve. It is a very important structure in the human eye. There is emerging evidence that estrogen metabolism has a vital role in the pathogenesis of primary open-angle glaucoma (POAG). In a study done showed that among women ≥ 65 years, early age of menopause (≤ 45 years) was associated with an increased risk of POAG (Hulsman *et al.*, 2001), whereas later age of onset of menopause (≥ 54 years) was associated with a decreased risk of POAG (Pasquale *et al.*, 2007). Researchers found that retinal ganglion cells express estrogen receptors (Munaut *et al.*, 2001), and estrogen has beneficial effects in animal models of neurodegenerative disorders, including glaucoma (Carroll *et al.*, 2010; Morissette *et al.*, 2008). Therefore, in menopause, the decline in estrogen level removes the protective effect of estrogen on retinal ganglion cells, causes RNFL thinning and increases the risk of patients to POAG.

Besides this, retinal ganglion cells are vulnerable to oxidative stress which generates reactive oxygen species. This leads to tissue hypoxia and further degeneration of the retinal ganglion cells (Munemasa and Kitaoka, 2012). Estrogen which has anti-oxidant properties removes this unwanted effect and protects the retinal ganglion cells and prevents RNFL thinning (Pasquale *et al.*, 2007).

In another study comparing the sex specific differences in RNFL thinning following acute optic neuritis, it was found that men had worse RNFL thinning that women after optic neuritis. On this aspect, estrogen has been shown to increase retinal blood flow and protect the RNFL in animal and clinical models of optic nerve injury (Deschenes *et al.*, 2010). In an experimental model of Leber hereditary optic neuropathy, administration of 17β-estradiol activated mitochondrial biogenesis and improved energetic competence, showing the protective effects of estrogen that may explain the higher prevalence of Leber hereditary optic neuropathy in men relative to women (Giordano *et al.*, 2011).

Based on the assessment of RNFL thickness between gender, various studies done in different countries showed no obvious difference in RNFL thickness between the gender (Alasil *et al.*, 2013; Budenz *et al.*, 2007; Mansoori *et al.*, 2012). However, one study done among Chinese population showed there was significant difference in the RNFL in the nasal quadrant between males and females and the thickness was higher in males (Qu *et al.*, 2014).

So far, there is very limited data on the normative data of RNFL thickness in post-menopausal women compared to reproductive age group women to assess whether there is an effect of female reproductive hormone on RNFL thickness. In Atas *et al*. (2014) study evaluating RNFL thickness between post-menopausal women and reproductive age group women, there was no significant difference found in the peripapillary RNFL thickness parameters between post-menopausal women and control group.

1.2.2.2.3 Optic Nerve Head (ONH) Parameters

Optic nerve head (ONH) is a small blind spot on the surface of the retina, located about 3 mm to the nasal side of the macula. It is the point where the retinal nerve fibers leave the eye and become part of the optic nerve. It is the only part of the retina that is insensitive to light. At the center the porus opticus marks the point of entrance of the central artery and vein of the retina. Evidence supports the hypothesis that estrogen deficiency is involved in the patho-physiology of optic nerve aging and glaucomatous neurodegeneration through several mechanisms (Vajaranant and Pasquale, 2012).

In relation to the mechanical theory, estrogens may regulate IOP by influencing the aqueous production and outflow systems through receptors in the ciliary epithelium (Altintas *et al.*, 2004; Ogueta *et al.*, 1999). In menopause, the decline in estrogen causes this regulation to be affected. With regards to the vascular theory, estradiol regulates smooth muscle tone and vascular resistance and augments the activity of endothelial-based nitric oxide synthase (NOS3) (Kang *et al.*, 2010). Specifically, estradiol may influence perfusion of the optic nerve, retinal ganglion cells, and their supporting structures. This effect has been demonstrated in animal models and clinical studies (Deschenes *et al.*, 2010; Harris *et al.*, 2000). A 22–45% increase in perfusion to the retina was reported in ovariectomized rats treated with estradiol compared to control ovariectomized rats treated with vehicle (Deschenes *et al.*, 2010). In humans, aging and age-related decreases in female sex hormones negatively affect ocular blood flow. In addition to clinical evidence and animal models, epidemiologic studies have also demonstrated an

association between decreased estrogen exposure and the risk for developing glaucoma later in life (Hulsman *et al.*, 2001; Nirmalan *et al.*, 2004).

Several studies done on ONH parameters showed there exists difference in ONH parameters between males and females (Hermann *et al.*, 2004; Ramrattan *et al.*, 1999; Tariq *et al.*, 2012; Zhang *et al.*, 2014). A study done among healthy adults in Kelantan also showed variation in ONH parameters between males and females (Jusoh *et al.*, 2011). These differences suggest a possible role of reproductive hormonal influence on ONH parameters. However, till date, there's no normative data regarding ONH measurement in post-menopausal women