# EVALUATION OF RETINAL NERVE FIBER LAYER AND MACULA THICKNESS, COLOUR VISION AND ELECTRORETINOGRAM IN PATIENTS WITH ACNE VULGARIS TREATED WITH SYSTEMIC ISOTRETINOIN

**DR. NURUL ZULAIKHA BINTI WAHAB**

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF THE MASTER OF MEDICINE (OPHTHALMOLOGY)

UNIVERSITI SAINS MALAYSIA

2021

**DISCLAIMER**

I hereby certify that the work in this dissertation is my own except for the quotations, some figures and summaries which have been duly acknowledged. I declare that I have no financial interest in the instrument and the computer software used in this study.

---

Dr. Nurul Zulaikha Wahab

Date: 28 February 2021

## ACKNOWLEDGEMENT

First and foremost, I am thankful to Allah, for making it possible for me to complete this thesis, I am also grateful to my parents who have given their relentless encouragement, love and sacrifices, without which, I would not be where I am today.

I wish to express my utmost gratitude to my supervisor, Dr. Khairy Shamel Sonny Teo, for his patience, support and invaluable guidance. You see my flaws, and yet you persevere, inspiring me as to be the best that I can be. You are an excellent mentor and an inspiring role model.

I am thankful to my co-supervisor, Dr. Wan Noor Hasbee Wan Abdullah, Department of Dermatology, Hospital Raja Perempuan Zainab II for his assistance and advice conducting this study. My sincere appreciation as well to all the lecturers and support staff in the Department of Ophthalmology and Visual Sciences for their help in the completion of this thesis. Last but not least, special mention for Cik Suhana Mohd Noor who worked tirelessly to help me complete the thesis.

# TABLE OF CONTENT

**LIST OF TABLES**

**LIST OF FIGURES**

**ABSTRAK**

**Pengenalan**

Isotretinoin (13-cis-retinoid acid) adalah retinoid sintetik yang digunakan dalam merawat jerawat jenis *acne vulgaris* yang tidak dapat dirawat dengan rawatan-rawatan yang lain. Keberkesanan isotretinoin yang amat tinggi dalam merawat *acne vulgaris*, menjadikan penggunaannya sangat meluas. Walaubagaimanpun, isotretinoin mempunyai profil kesan sampingan yang luas termasuklah ke atas mata dan penglihatan. Terdapat beberapa kajian yang menunjukkan kesan sampingan isotretinoin terhadap saraf mata (*retinal nerve fiber layer* dan *macula layer*) dan juga fungsi penglihatan.

**Objektif**

Kajian ini bertujuan untuk mengesan kesan sampingan isotretinoin ke atas mata dengan melihat perubahan ke atas saraf mata [*retinal nerve fiber layer* (*RNFL*) dan *macula*] dan perubahan ke atas fungsi [penglihatan warna dan *electroretinogram* (*ERG*)].

**Kaedah**

Kajian ini adalah kajian kohort pemerhatian prospektif yang melibatkan pesakit yang menghidapi *acne vulgaris* yang diberikan rawatan ubat makan isotretinoin. Kajian ini dijalankan daripada Jun 2018 sehingga Mei 2021 di Hospital Universiti Sains Malaysia dan Hospital Raja Perempuan Zainab II. Semua pesakit yang terlibat telah menjalani pemeriksaan mata yang lengkap. Perubahan ketebalan saraf mata (*retinal nerve fibre layer* and *macula*) diukur menggunakan mesin *optical coherent topography* (OCT), perubahan fungsi mata diukur melalui penglihatan menggunakan ujian *Farnworth-Munsell 100 Hue* dan

*ERG*. Kesemua ujian ini dilakukan sebelum memulakan rawatan isotretinoin dan pada tiga bulan selepas memulakan rawatan. SPSS Inc Versi 26 digunakan untuk analisis statistik.

**Keputusan**

Seramai 40 pesakit yang dirawat menggunakan ubat isotretinoin 20 mg sehari telah menyertai kajian ini. Purata umur pesakit yang menyertai kajian ini adalah 26.88(7.16) tahun dengan julat umur 17-43 tahun. Ukuran ketebalan saraf mata *RNFL* dan *macula* sebelum memulakan rawatan masing-masing adalah 93.88 (11.08) µm dan 300.94(14.66) µm. Manakala purata ukuran ketebalan *RNFL* dan *macula* pada tiga bulan selepas rawatan masing-masing adalah 94.35(10.11) µm dan 301.42(15.65) µm. Statistik menunjukkan tiada perbezaan yang ketara di antara dua ukuran tersebut ($p>0.05$). Skor penglihatan warna sebelum rawatan adalah 76.30(32.27) dan 74.15(24.17) pada tiga bulan selepas memulakan rawatan. Amplitud gelombang *a* dan gelombang *b* didalam ERG sebelum memulakan rawatan adalah masing-masing 269.24(57.00) µV dan 450.48(70.07) µV. Manakala amplitud gelombang *a* dan gelombang *b* pada tiga bulan selepas memulakan rawatan adalah masing-masing 268.68(55.13) µV dan 448.40(69.91) µV. Tiada perubahan statistik yang ketara dapat diperhatikan didalam penglihatan warna dan gelombang *ERG* pada tiga bulan selepas memulakan rawatan ($p<0.05$).

**Kesimpulan**

Tiada perubahan statistik yang ketara di dalam purata ketebalan lapisan *RNFL* dan *macula*, penglihatan warna dan *ERG* di kalangan pesakit *acne vulgaris* pada sebelum dan selepas tiga bulan rawatan sistemik isotretinoin.

## ABSTRACT

### Introduction

Isotretinoin (13-*cis*-retinoic acid) is a synthetic retinoid that is used specially for treating acne vulgaris that does not respond to other therapies. Isotretinoin is an extremely effective systemic medication for acne vulgaris which is widely used. However, isotretinoin is also known to have a broad side effect including ocular toxicity. Some studies have shown its effect on retinal nerve fiber layer (RNFL) and ganglion cell layer and visual functions.

### Objectives

This study was performed to evaluate RNFL and macular thickness, colour vision and electroretinogram (ERG) on systemic isotretinoin treatment in acne vulgaris patients.

### Methods

Prospective observational cohort study involving patients with moderate to severe acne vulgaris on systemic isotretinoin was conducted at Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II from June 2018 till May 2021. All patients had to undergo a complete ophthalmologic examination. RNFL and macula thickness analysis using optical coherence topography (OCT), colour vision analysis using Farnsworth-Munsell 100 Hue test and ERG were performed before the start of treatment and at three months of treatment. Statistical analysis was done using SPSS Inc Version 26.

**Results**

Forty patients who were treated with 20 mg daily oral isotretinoin were enrolled in this study. The mean age of the patients was 26.88 (7.16) years (which is between 17-43 years). Measurements of mean RNFL and macula thickness before starting isotretinoin treatment was 93.88 (11.08) µm and 300.94 (14.66) µm respectively, while the mean RNFL and macula thickness at 3 months of treatment was 94.35(10.11) µm and 301.42(15.65) µm respectively. Measurements showed no statistically significant change between two measurements ($p>0.05$). The colour vision score was 76.30 (32.27) before starting treatment and 74.15 (24.17) at 3 months of treatment. The amplitude of *a* wave and *b* wave in ERG before starting treatment was measured as 269.45(57.00) µV and 450.48(70.07) µV respectively. Whereas the amplitude of a wave and b wave at 3 months of treatment was measured as 268.68 (55.13 µV) and 448.40 (69.91) µV respectively. No statistically significant differences were observed in the colour vision score and ERG amplitude at three months of treatment ($p>0.05$).

**Conclusion**

There was no statistically significant difference in mean RNFL and macula thickness, colour vision and ERG of acne vulgaris patients before and at three months of systemic isotretinoin treatment.

# Chapter 1
# Introduction

# 1. INTRODUCTION

## 1.1 ACNE VULGARIS

### 1.1.1 Pathogenesis of Acne

Acne vulgaris is a multifactorial disease involving four primary pathogenic factors producing acne lesion. The pathogenesis involves sebum production by the sebaceous gland, *Propionibacterium acnes* follicular colonization, alteration in the keratinization process and release of inflammatory mediators into skin (Thiboutot et al., 2009).

Sebaceous gland has a significant role in hormonal induced aging skin by regulating endocrine functions of the skin independently (Thiboutot et al., 2009). The sebaceous gland is highly responsive to the stimulation of androgens. Stimulation of androgens causes the gland to become hypertrophy and thus secretes sebum into follicular canal (Winston and Shalita, 1991). Sapienic acid, a free fatty acid in sebum that mediates inflammation by chemotactic properties towards polymorphonuclear leukocytes and monocytes in the presence of bacteria (Thiboutot et al., 2009; Winston and Shalita, 1991). Free fatty acid may provide the primary stimulus for the retention follicular hyperkeratosis (Winston and Shalita, 1991). Sebum also provides a favourable environment for the proliferation of P. *acnes* (Winston and Shalita, 1991).

P. *acnes* plays an important and critical role in the generation of inflammatory lesions. P. *acnes* contains a soluble factor that can induce proinflammatory cytokine production in

human monocytic cell lines (Thiboutot et al., 2009). The activity of soluble factor is dependent on the presence of CD14, a so-called pattern recognition receptor of lipopolysaccharide and other lipid-containing ligands (Thiboutot et al., 2009). P. *acnes* product induced the synthesis of cytokines (tumor necrosis factor-α and IL-1β) in the cell lines through TLR-2, a protein known as toll (Thiboutot et al., 2009). This toll protein could trigger a signaling cascade that activates nuclear factor-κB (Thiboutot et al., 2009). These inflammatory cytokines activate activator protein (AP)-1 transcription factor, thus induce MMP genes whose product degrade and alter dermal matrix (Thiboutot et al., 2009). Subsequently, the intrafollicular contents (keratinous debris, lipids, hair and P. *acnes*) extruded into dermis (Winston and Shalita, 1991). This extrusion causing formation of pustule, nodule and cyst (Winston and Shalita, 1991).

Peroxidation of sebum lipids in human keratinocyte cell can activate inflammatory mediators, including IL-6 and lipoxygenase (Ottaviani et al., 2006). Oxidized squalene can also stimulate keratinocytes hyperproliferation, suggesting that this lipid may be partly responsible for comedo formation (Thiboutot et al., 2009). Lipoperoxidase is also able to exert a proinflammatory effect on pilosebaceous duct by producing leukotriene B4, which is a power chemoattractant that can recruit neutrophils and macrophages, and stimulate production of proinflammatory cytokines (Alestas et al., 2006; Thiboutot et al., 2009). Matrix metalloproteinase (MMPs) which includes collagenase, gelatinases, stromelysins, and matrilysisn, have a prominent role in inflammatory matrix remodeling and proliferative skin disorders. Sebum induces several MMPs that originate in keratinocytes and sebocytes (Papakonstantinou et al., 2005).

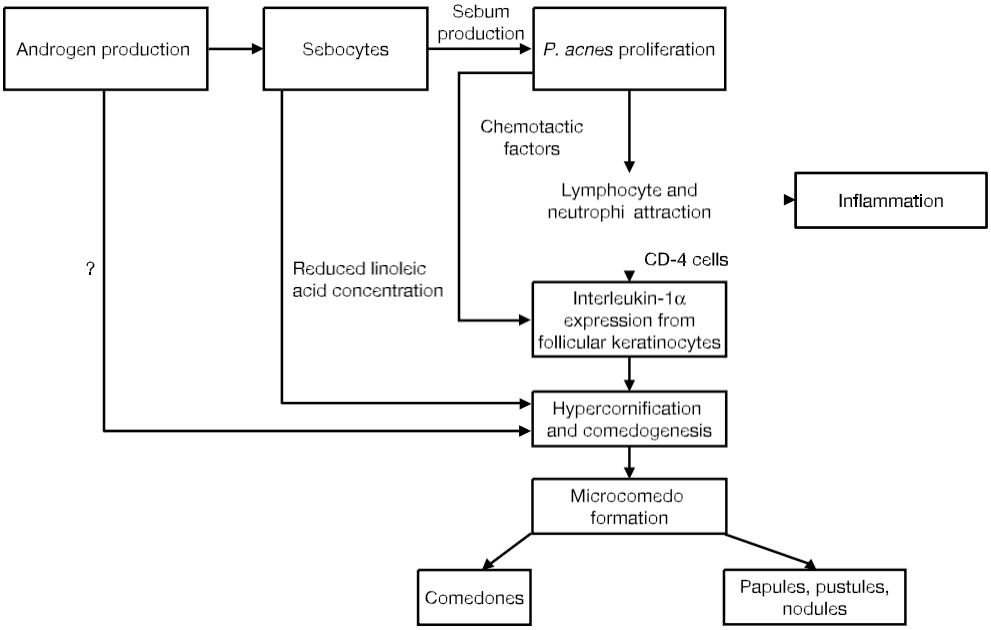


Figure 1.1 Pathogenesis of acne (Leyden, 2003)

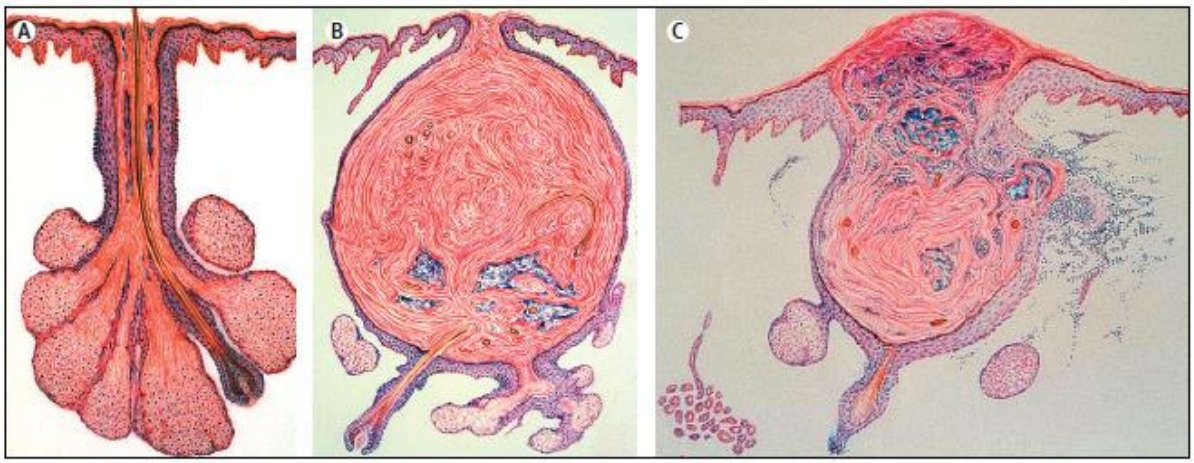


Figure 1.2: Histological structure of (A) Normal sebaceous follicle, (B) comedo, (C) inflammatory acne lesion with rupture of follicle wall and secondary inflammation (Williams et al., 2012).

### 1.1.2 Epidemiology of Acne

Acne is most common problem in adolescents, affecting approximately 85% of teenagers (Knutsen-Larson et al., 2012). The average age for onset of acne is 15 – 17 years (Bhate and Williams, 2013; Hanisah et al., 2009; Williams et al., 2012). Although acne is most prevalent in this group, it can manifest at any time during life, even as early as the neonatal period (Winston and Shalita, 1991). Study by (Hanisah et al., 2009) showed acne affecting 71.1% of boys and 64.6% of girls. Severity of acne in boys correlates with pubertal maturation, as they have higher androgen levels and oilier complexion (Hanisah et al., 2009). However, by increasing age, acne is more common in women (Hanisah et al., 2009; Knutsen-Larson et al., 2012). Adult acne is usually represented by the chronic persistence from the adolescence age, not during new onset (Knutsen-Larson et al., 2012). There are a few modifiable factors that can alter acne risk such as genetic, cigarette smoking, diet and environment (Knutsen-Larson et al., 2012).

### 1.1.3 Clinical Features and Grading System

Acne is a pleomorphic disorder of variable course and anatomical distribution (Doshi et al., 1997). Diagnosis of acne is typically made by clinical evaluation by using grading scale, lesion counting and photographic methods (Ministry of Health Malaysia, 2012). There are various acne grading system existed to categorise the severity of acne (Doshi et al., 1997). Comprehensive Acne Severity Scale (CASS) is a tool that has been recommended by (Ministry of Health Malaysia, 2012) as it is accurate, reproducible, rapid and simpler to be used in clinical practice.

| GRADE* | | DESCRIPTION |
|---|---|---|
| Clear | 0 | No lesions to barely noticeable ones. Very few scattered comedones and papules. |
| Almost clear | 1 | Hardly visible from 2.5 metre away. A few scattered comedones, few small papules and very few pustules. |
| Mild | 2 | Easily recognisable; less than half of the affected area is involved. Many comedones, papules and pustules. |
| Moderate | 3 | More than half of the affected area is involved. Numerous comedones, papules and pustules. |
| Severe | 4 | Entire area is involved. Covered with comedones, numerous pustules and papules, a few nodules and cyst. |
| Very severe | 5 | Highly inflammatory acne covering the affected area, with nodules and cyst present. |

Table 1.3: Grading Acne Severity based on Comprehensive Acne Severity Scale (CASS) (Ministry of Health Malaysia, 2012).

### 1.1.4 Management of Acne

Management of the acne is based on the severity of the acne and predominant lesions based on Grading Acne Severity (Ministry of Health Malaysia, 2012). The aim of acne management is to induce clearance of lesions, to maintain remission and prevent relapse, and avert physical and psychological complications (Ministry of Health Malaysia, 2012). Modalities of treatment consist of pharmacological and non-pharmacological means. Oral isotretinoin is the preferred treatment for severe acne or acne that does not responded to other modalities (Leyden, 2003; Ministry of Health Malaysia, 2012; Thiboutot et al., 2009; Winston and Shalita, 1991; Zaenglein, 2018).

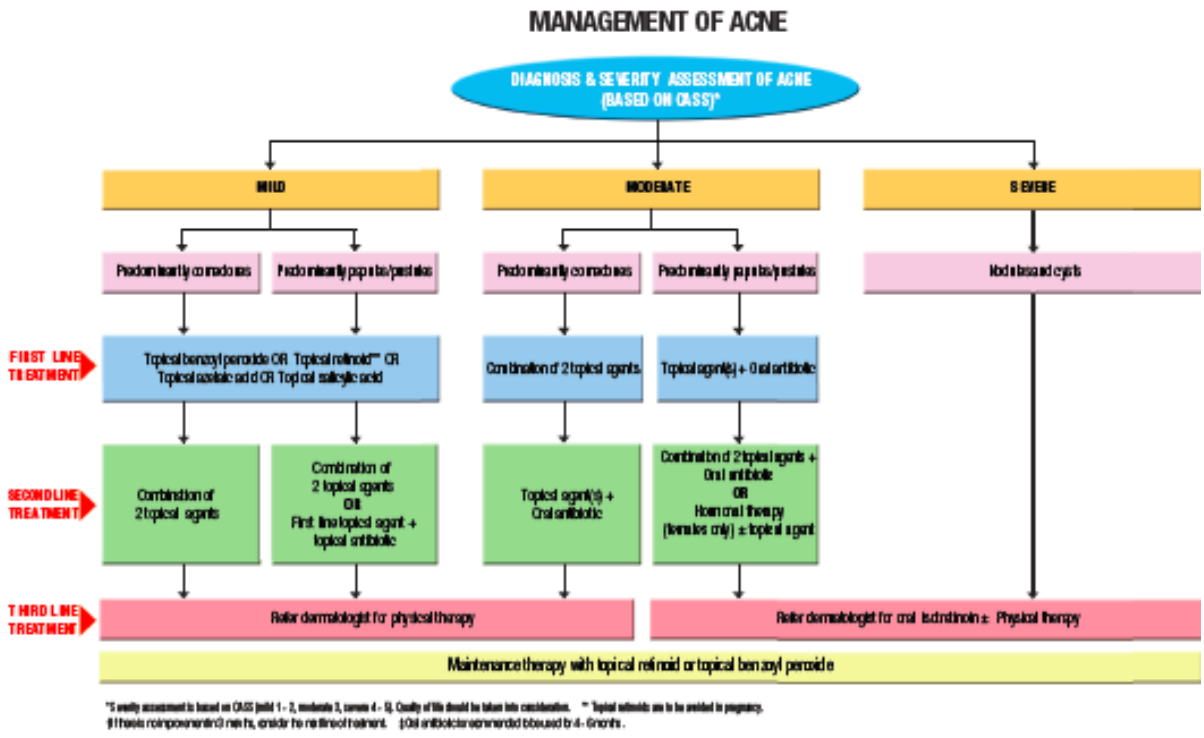


Figure 1.4: Algorithm on Management of Acne (Ministry of Health Malaysia, 2012)

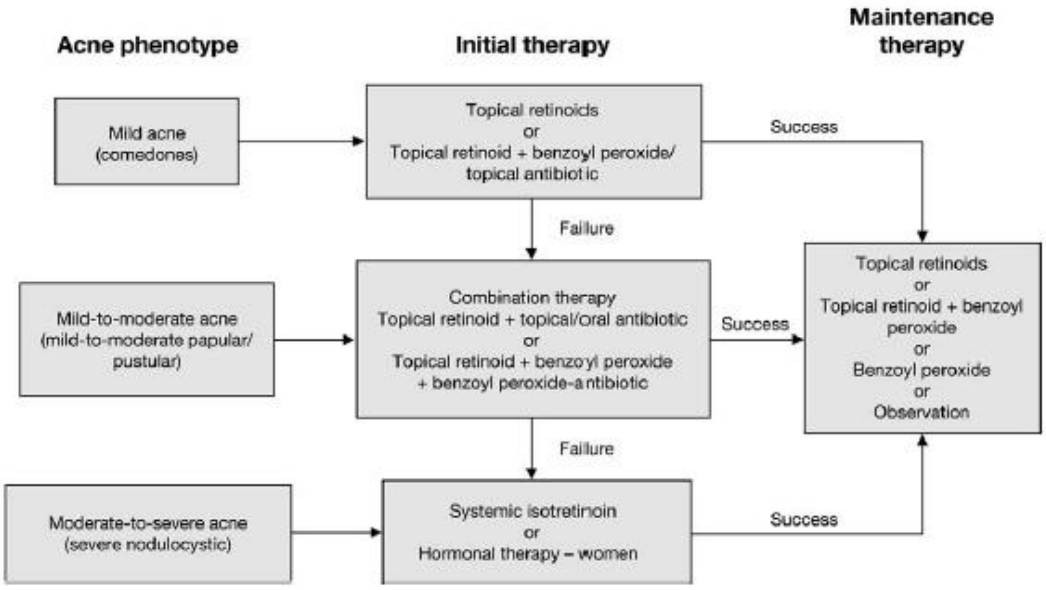


Figure 1.5: Proposed algorithm for the management of acne vulgaris in dermatology clinical practice (Leyden, 2003)

## 1.2 ISOTRETINOIN

### 1.2.1 Vitamin A Metabolism

Vitamin A is not produced by the human body. Therefore, it is usually obtained from dietary regiments (Davidovici et al., 2007). Vitamin A is found in three interconvertible forms; retinol (vitamin A alcohol) which is the main dietary, transport and storage form, retinal (vitamin A aldehyde) which is necessary for visual function, and retinoic acid (vitamin A acid) which is biologically active ligand that is binded to intracellular retinoid receptors (Bagatin and Costa, 2020).

Vitamin A plays a very important role in the metabolism of visual pigments to maintain the visual functions (Safran et al., 2015). The metabolism of vitamin A in retina is known as 'visual cycle', Figure 1.6 (Safran et al., 2015). The first step in the visual cycle is the absorption of light by photopigment located in photoreceptor. Human eyes contain two types of photoreceptors which are rods and cones (Wang and Kefalov, 2011). The absorption of light by photopigments generates a hyperpolarization of the receptor, which in turn result in the electrical message being passed from retina to the optic nerve (Safran et al., 2015). The absorption of light leads to the splitting of photopigment, e.g., rhodopsin into two molecules, opsin and retinaldehyde in the all-*trans* configuration. Since the rhodopsin has been split and destroyed, other rhodopsin must be synthesised (Safran et al., 2015). The retinal that splits off from the rhodopsin exist in inactive all-*trans* form (Safran et al., 2015). It has to be activated into 11-*cis* configuration to initiate visual excitation. Therefore, all-*trans* must be isomerised. The isomerization of all-trans to 11-cis retinoids is a critical step in metabolising

vitamin A for vision (Safran et al., 2015). All-trans-retinal is transformed to all-trans-retinol, then isomerised to 11-cis-retinol, oxidised to the corresponding retinaldehyde, and finally bound to opsin to reconstitute rhodopsin (Safran et al., 2015). The isomerisation is facilitated by isomerase and 11-cis-retinaldehyde dehydrogenase (Safran et al., 2015). High concentration of retinol in the retina can affect the stability of membrane, retinol is esterified stored in the form of retinyl ester. The development of retinoid biosynthetic system discovered that isotretinoin and tretinoin are powerful inhibitors of retinol dehydrogenase, and thus prevent rhodopsin regeneration (Safran et al., 2015).

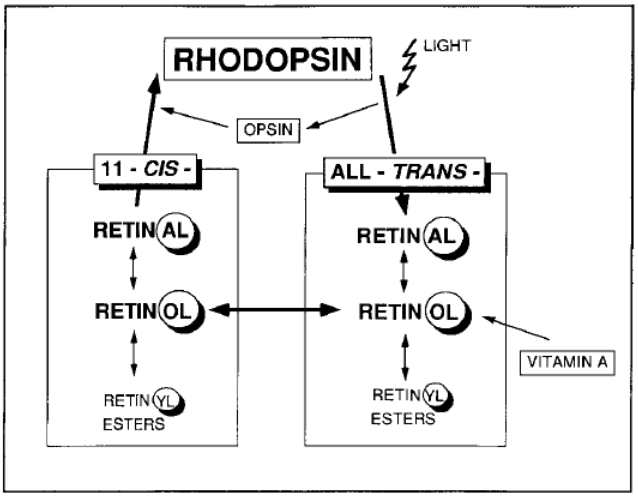


Figure 1.6: Diagram of visual cycle (Safran et al., 2015).

### 1.2.2 Isotretinoin and Mechanism of Action

Isotretinoin (13, cis-retinoid acid (13-Cra)) is a synthetic retinoid that is used especially for severe cystic acne that does not respond to other therapies (Ucak et al., 2014). Retinoids are

modification of vitamin A molecules (Yob and Pochi, 1987). Isotretinoin is the first generation which is stemmed from manipulation of the polar end group and polyene side chain (Yob and Pochi, 1987). It was first approved as treatment for severe acne by the US Food and Drug Administration (FDA) in 1982. Isotretinoin revolutionised the management of acne vulgaris and various skin disorder when it was first introduced in the 1980s. Unlike other therapies of acne, isotretinoin is the only therapy that has an impact on all of the major etiological factors implicated in acne by reducing, sebaceous gland size and decrease sebum production, which in turn inhibits P. *acnes* and its ability to elicit inflammation (Brelsford and Beute, 2008; A. Layton, 2009). Retinoids are able to inhibit activator protein (AP)-1, thus reduces MMP genes expression (Thiboutot et al., 2009). Retinoids also can induce monocytes to develop into $CD209^{+}$ macrophages that phagocytose P. *acnes* (Thiboutot et al., 2009).

Since isotretinoin introduction, numerous case reports have documented significant improvement and prolonged clinical remissions sustained while on the therapy. The effectiveness of oral isotretinoin treatment for acne vulgaris has been reported to be as high as 70-89% (Cunliffe et al., 1997).

Even though isotretinoin is an effective medication in treating acne vulgaris, it also has a broad side-effect. Ocular side effects have been recorded in isotretinoin treatment. In particular, blurred vision, keratitis, corneal opacities, blepharoconjunctivitis, sicca, decreased dark adaptation, photophobia, retinal abnormalities and neurologic disorder such as idiopathic intracranial hypertension, optic neuritis, visual field defects and cortical

blindness have been reported among patients who had undergone systemic isotretinoin treatment (Fraunfelder et al., 2001).

While most of these adverse effects are similar to those seen with excessive vitamin A administration, the type and severity of these effects can differ between retinoids, which is also influenced by the dosage usage and duration of treatment (Yob and Pochi, 1987). The symbiotic relationship between vitamin A and retinoid toxicity is further exemplified by the fact that 70 mg of a retinoid, such as isotretinoin is equivalent to 250 000 IU of vitamin A, which is quite close to the early toxic levels in humans(Yob and Pochi, 1987). Recommended daily intake of vitamin A in adult is 5 000 to 10 000 IU (Hathcock et al., 1990).

The suggested dose of isotretinoin to treat acne vulgaris is 0.5-1.0 mg/kg per day, administered bid (Haider and Shaw, 2004). The treatment period is approximately between 16-24 weeks and the total cumulative dose usually exceeds 120-150 mg/kg (Haider and Shaw, 2004). The low dosage treatment is suggested to be 0.15-0.40 mg/kg per day, with a total cumulative dose of less than 120 mg/kg (Bagatin and Costa, 2020). With low dose treatment, the incidence of adverse effects is reported to be low and the there is a reduction in the cost of treatment (Bagatin and Costa, 2020; Cumurcu et al., 2009). Despite that, lower dosage administered affects the success rate of the treatment (Haider and Shaw, 2004).

A suggested dose of 20 mg/day for patients with severe acne or who have failed to respond to other modalities (Ministry of Health Malaysia, 2012). A dose of 20 mg/day for six months showed complete or almost complete remission in 94.8% of patients aged 12-20 years old and 92.6% aged 21-35 years old (Ministry of Health Malaysia, 2012). Failure of treatment was 5.2% and 7.4% respectively (Ministry of Health Malaysia, 2012). Within the four-year

follow up period, relapses occurred in 3.9% of patients aged 12-20 years old and 5.9% for patients aged 21-35 years old (Ministry of Health Malaysia, 2012). Based on the recommendation, a daily dose of 20mg/day is practised in Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II. The total duration of isotretinoin treatment for acne vulgaris in these two study centres were varied as it was decided by the dermatologists based on their clinical judgment. But most of the moderate to severe acne vulgaris cases were treated between the duration of three to six months.

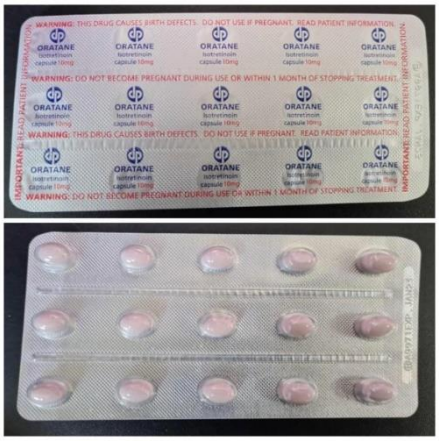


Figure 1.7: Caps Isotretinoin 10 mg used in Hospital Raja Perempuan Zainab II.

## 1.3 RETINAL NERVE FIBER LAYER

RNFL is formed by axons of retina ganglion cells and macula ganglion cells, that is located in the inner retina (Gümüş and Öner, 2015). The axons form the ganglion cells converge at the optic disc to form the optic nerve (Varma et al., 1996). Approximately 82% of tissues in the RNFL compromises of neural/axonal tissues (Varma et al., 1996). These layers transmit impulses from photoreceptors to central visual cortex via optic nerves (Gümüş and Öner, 2015).

Retina is particularly susceptible to damage from drug exposure, and isotretinoin is one of the systemic drugs that can cause retinopathy (Feigl et al., 2000; Nencini et al., 2008; Weleber et al., 1986). RNFL thickness has the potential to be the indicator to measure toxic effect of ocular system (Gümüş and Öner, 2015; Lawthom et al., 2009). Changes in RNFL thickness has an effect on visual acuity (Gümüş and Öner, 2015). Changes in RNFL thickness can be measured using optic coherence tomography (OCT) (Gümüş and Öner, 2015; Lawthom et al., 2009; Vieira et al., 2015). OCT is a high-resolution cross-section imaging that is used to measure retinal and macula thickness and its layers (Huang et al., 1991). The Spectral-Domain OCT (SD OCT) measures RNFL thickness at peripapillary RNFL circle in four quadrant which are superior, inferior, nasal and temporal quadrants (Demirok et al., 2017; Dichtl et al., 1999; Varma et al., 1996; Yılmaz et al., 2017). The peripapillary RNFL thickness is automatically calculated by the SD OCT, and provides a global average and the average thickness of each quadrants (Yanni et al., 2013).

In a study conducted by (Ucak et al., 2014), they have found significant decrease in thickness of RNFL all temporal quadrants as compared to baseline. On the other hand, (Sekeryapan et al., 2013)did not find significant difference between pre- and post-treatment of RNFL and macula ganglion cell layer thickness in patients who received isotretinoin therapy for 6.5 months on spectral-domain OCT. (Kaptı et al., 2013) also did not find significant difference in post-treatment RNFL thickness compared with pre-treatment values in patients who had received systemic isotretinoin therapy during 6 months of follow up.

In a study conducted by (Ucak et al., 2014), there was no significant difference between pre- and post-treatment mean RNFL thickness measured by OCT in their patients who had received isotretinoin therapy with a mean duration of 5.4 months. However, significant decrease in temporal inferior quadrant of RNFL was detected. The authors reported a possible toxic effect of isotretinoin on RNFL, especially in the temporal layer. It is suggestive that the evaluation of temporal inferior quadrant of RNFL may be important to detect adverse effects of isotretinoin therapy in retina.

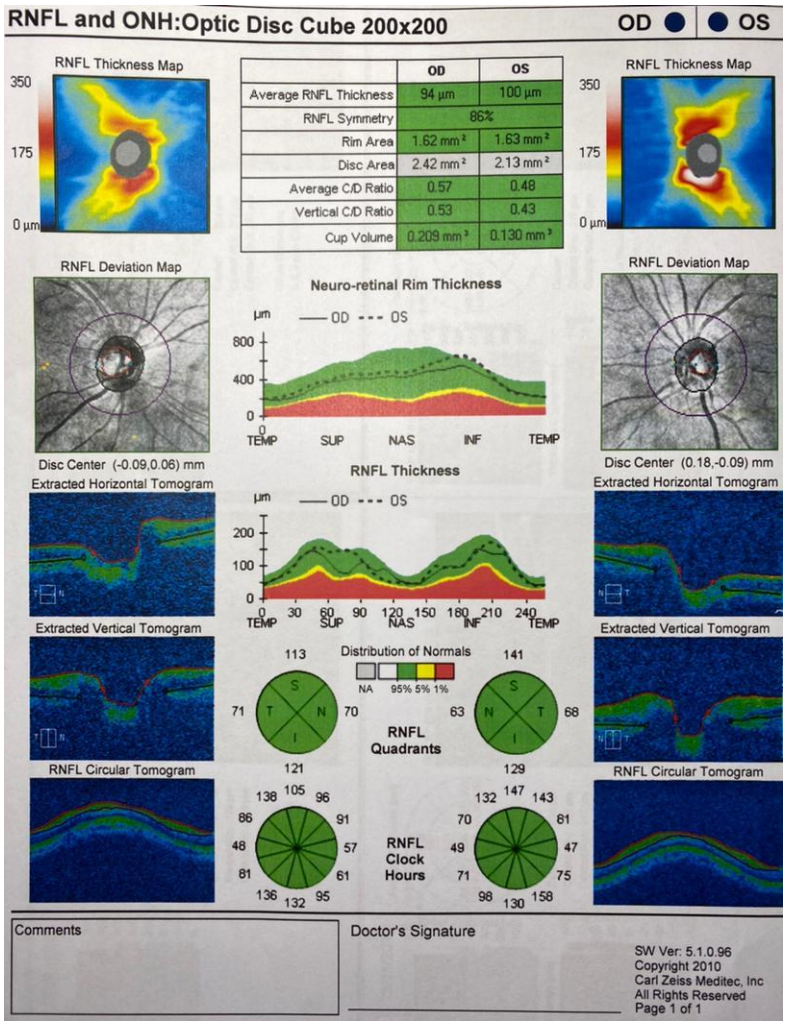


Figure: RNFL thickness measurement using SD OCT.

## 1.4 MACULA

Macula is a structure that plays a crucial part in central vision, visualisation of fine details and image resolution. Macula is a round area in the posterior pole of retina, which measures between 5 – 6 mm in diameter, centred 4.0 mm temporal and 0.8 mm inferior to the centre of optic disc (Salmon, 2020). Macula subserves the central 15-20° of visual field (Salmon,

2020). Approximately 50% of the total retinal ganglion cells of RNFL synapse in the central 5 mm of the macula (Demirok et al., 2017). Therefore, macula ganglion cell complex thickness analysis is sensitive to detect the quantity of ganglion cell damage (Demirok et al., 2017).

Retinal ganglion cells contain melanopsin, which is very similar to other opsin pigments and melanopsin use the same retinaldehyde chromophore (Kocamis and Acer, 2017). Therefore, isotretinoin may exert an inhibitory effect on the chromophore regeneration in retinal ganglion cells similar to what is seen in rod and cone photoreceptors (Kocamis and Acer, 2017).

In a study conducted by (Yılmaz et al., 2017), it was found that there is a significant decrease of macular thickness in superior, nasal and temporal outer quadrants at second and third months after commencement of therapy, while significant decrease was being observed in superior inner quadrant at first, second and third months after beginning of therapy as compared to baseline. They concluded that oral isotretinoin therapy could cause regional thinning in RNFL and macula, directly visible part of central nervous system. (Kocamis and Acer, 2017) conducted a study on patients using systemic isotretinoin (1mg/kg/day) for 2 – 3 months, it showed there was no statistical difference in RNFL thickness and macula thickness.

The macula thickness were assessed by high speed Spectralis SD OCT and classified into nine different regions (Yanni et al., 2013). The retinal thickness map was used to determine the numeric averages of thickness for 5 subfield within the Early Treatment Diabetic Retinopathy Study grid (Demirok et al., 2017; Yanni et al., 2013; Yılmaz et al., 2017).

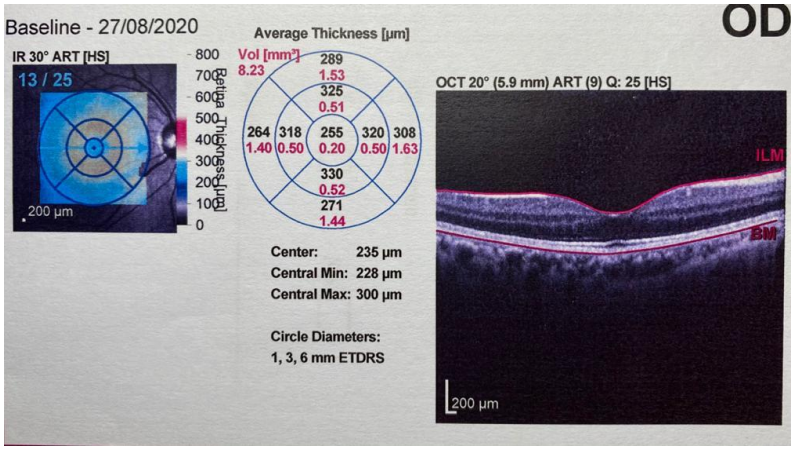


Figure 1.8: Macula thickness measurement based on Early Treatment Diabetic Retinopathy Study grid using Spectralis SD OCT.

## 1.5 COLOUR VISION

Normal human colour vision is trichromatic, which means that any colour can be produced with a mixture of three judiciously chosen primary colours (Simunovic, 2010). Cone photoreceptor is the physiological substrate of colour vision (Simunovic, 2010). It contains three classes which are blue short wavelength cones, green medium wavelength cones and red long wavelength cones (Simunovic, 2010). Different class of cones contains different

types of photopigment. Photopigment of cones comprised of two components which is opsin and 11-*cis* retinal (a derivative of dietary vitamin A) (Simunovic, 2010). It is the photopigments that are responsible for absorbing light to enter into visual cycle cascade for vision (Safran et al., 2015; Simunovic, 2010).

Colour vision is tested using Farnsworth-Munsell (FM) 100 Hue Test. FM 100 Hue test is a sensitive tool for colour discrimination (Foote et al., 2014; Zahiruddin et al., 2010). It has the ability to distinguish people with normal colour vision into classes of superior, average, and low discrimination abilities, and in people who have congenital colour deficiencies (Foote et al., 2014; Zahiruddin et al., 2010). It gives indication of the axis of confusion which is along a blue-yellow or red-green axis or generalised colour vision (Foote et al., 2014; Zahiruddin et al., 2010). FM 100 Hue test is the standard assessment in detecting acquired colour vision deficiency (Foote et al., 2014).

(Oner et al., 2005) is the first series suggesting an association between isotretinoin and reversible decreased colour vision using FM 100 Hue test. The score of the FM 100 Hue test increases during treatment and it was statistically significant. But none of the patients complained of decreased colour vision. (Fraunfelder et al., 1985) reported there was a decreased in their colour vision between 3 -14 months after isotretinoin therapy. However, colour vison was not tested on those affected.

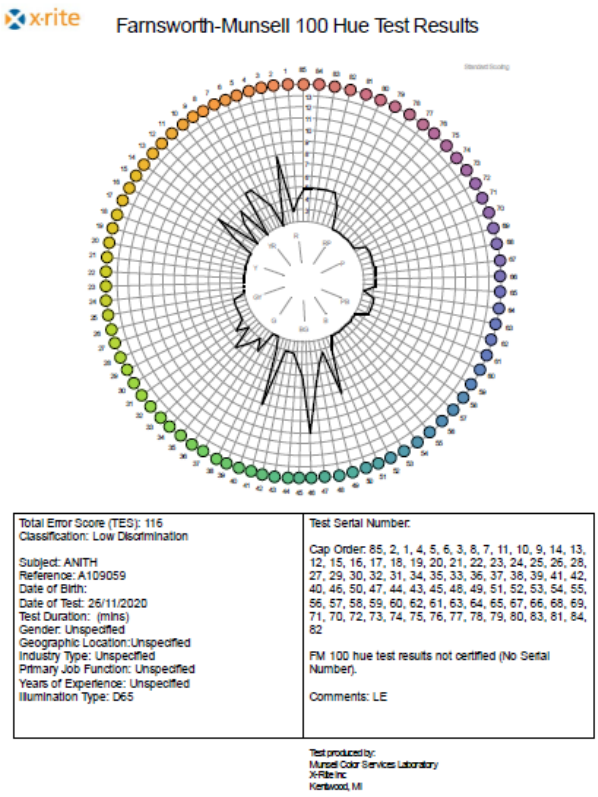


Figure 1.9: Farnworth-Munsell 100 Hue Test Result.

## 1.6 ELECTRORETINOGRAM

Electroretinogram (ERG) is a mass electrical potential generated by retina, evoked by a brief of flash light (Tsang and Sharma, 2018). ERG potentials are recoded by active electrode (a contact lens on cornea) and a reference electrode (at outer canthus) (Tsang and Sharma, 2018). ERG consist mainly of a negative *a* wave and followed by a positive *b* wave (Safran et al., 2015). The *a* wave is the initial downward deflection of recording generated by photoreceptors, while the upward deflection *b* wave is generated by Muller cells (Safran et al., 2015).

It is clinically important to differentiate between rod and cone functions as these two categories of photoreceptors serve different functions, and secondly because these two systems can selectively be affected by disease (Safran et al., 2015). Cone-pathway function for high visual spatial discrimination and colour vision is assess by photopic (light adapted) ERG while rod-pathway function for dark adaptation or night vision and movement perception is assessed by scotopic (dark adapted) ERG (Safran et al., 2015).

Nyctalopia or night blindness is a manifestation of vitamin A deficiency (Charakida et al., 2004). It has been postulated that the cause of night blindness may be due to competitive inhibition of ocular dehydrogenase of isotretinoin (Charakida et al., 2004). Inhibition of ocular hydrogenase which leads to local vitamin A deficiency, that reduces regeneration of rhodopsin which is imperative for rod photoreceptor function and visual cycle. This resulted in decreased dark adaptation, which is night blindness.

Objective evaluation using ERG has been found to be a useful diagnostic tool in evaluating ocular side effects of retinoids (Safran et al., 2015). Night blindness with objectively abnormal scotopic ERG had been reported with the usage of isotretinoin (Ellis and Krach, 2001). Study by (Brown and Grattan, 1989) on 12 patients treated with isotretinoin for 3 months showed significant drop in the a wave amplitude of scotopic ERG at 3 months of treatment. A study by (Fraunfelder et al., 2001; Weleber et al., 1986) observed of poor night vision, excessive glare sensitivity and abnormal dark-adaptation curves with elevations of cone-rod threshold in 3 out of 50 patients who had received systemic isotretinoin therapy. For one patient, ERG remained abnormal 6 months after cessation of therapy. (Weleber et

al., 1986) conducted a study on 50 patients treated with 1mg/kg/day, a closer look of isotretinoin showed abnormalities in dark adaptation on scotopic ERG.

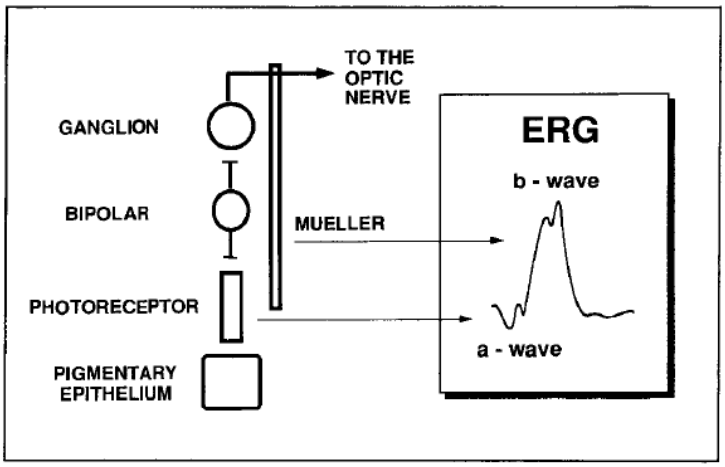


Figure 1.10: A schematic diagram of retina and its principal cells in normal ERG response. Photoreceptor generates the *a* wave and Muller cell generates the *b* wave. (Safran et al., 2015).

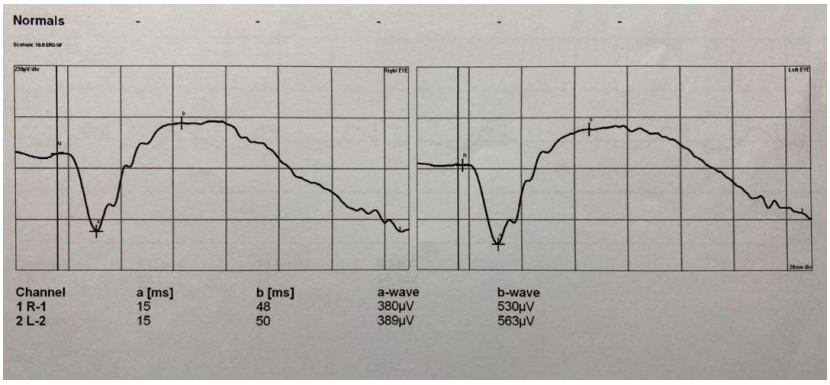


Figure 1.11: Scotopic ERG recording.

## 1.7 RATIONALE OF STUDY

The use of isotretinoin is increasing, it is imperative to find out the impact as well as possible changes it has on patient's visual function. Even though, the studies carried out to determine the effects of systemic isotretinoin therapy on RNFL and macula thickness, colour vision and ERG are limited and demonstrated conflicting results, the OCT, HM 100 Hue test and ERG have been found to have the sensitivity to identify the changes which were not detected during routine examination of visual function.

This study may provide insights on detecting early changes in isotretinoin induced visual toxicity. Outcomes of this study can be incorporated into the guidelines for starting the treatment of isotretinoin especially, in Malaysia. The OCT could be a useful tool to detect early anatomical changes in RNFL and macula, while HM 100 Hue test and ERG can be used to detect early physiological changes. Early detection of the changes may help in preventing the debilitating toxic optic neuropathy.

# Chapter 2
# Research Objectives

## 2.1 RESEARCH OBJECTIVES

### 2.1.1 GENERAL OBJECTIVES

To evaluate retinal nerve fibre layer and macular thickness, colour vision and ERG on systemic isotretinoin treatment in acne vulgaris patients.

### 2.1.2 SPECIFIC OBJECTIVES

1. To compare the mean RNFL thickness pre- and post-three months of systemic isotretinoin therapy in acne vulgaris patients.

2. To compare the mean macula thickness pre- and post-three months of systemic isotretinoin therapy in acne vulgaris patients.

3. To compare the colour vision score pre- and post-three months of systemic isotretinoin therapy in acne vulgaris patients.

4. To compare the amplitude ERG wave pre- and post-three months of systemic isotretinoin therapy in acne vulgaris patients.