EVALUATION OF VISUAL ACUITY AND MACULAR THICKNESS POST FOCAL LASER WITH AND WITHOUT SUPPLEMENTARY HONEY IN DIABETIC MACULAR OEDEMA

By:

DR. RAIHAN BT ABD RAHIM
MD (USM)

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DISCLAIMER

I hereby certify that the work in this dissertation is my own except for the quotations and summmaries which have been duly acknowledged.

Date: 28 November 2015

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(Dr Raihan bt Abdul Rahim)

(PUM 0123/10)

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ABBREVIATIONS

DM	diabetes mellitus
DMO	diabetic macular oedema
CSMO	clinical significant macular oedema
OCT	optical coherence tomography
CMT	central macular thickness
BCVA	best corrected visual acuity
LogMAR	logarithm of the minimum angle of resolution
HPT	hypertension
HPL	hyperlipidemia

ABSTRAK

Pengenalan

Diabetik makular edema adalah salah satu komplikasi diabetes pada mata yang boleh menyebabkan kebutaan. Rawatan bagi penyakit ini adalah terapi laser. Keradangan yang berpanjangan juga memainkan peranan penting di dalam penyakit diabetik makular edema. Madu tualang mengandungi anti-inflammasi dan anti-oksidan dan berkeupayaan sebagai terapi tambahan untuk merawat diabetik makular edema.

Objektif

Matlamat kajian ini adalah untuk membandingkan perbezaan ketajaman penglihatan terbaik LogMAR (LogMAR BCVA) dan ketebalan pusat makula (CMT) selepas 3 bulan rawatan menggunakan 2 kaedah berbeza iaitu gabungan rawatan terapi laser dan pemakanan madu tualang dan kaedah rawatan monoterapi laser ke atas pesakit diabetik makular edema.

Metodologi

Kajian ini merupakan kajian prospektif terkawal secara rawak yang dijalankan di Hospital Universiti Sains Malaysia antara April 2013 dan Ogos 2015. Pesakit yang menghidapi diabetik makular edema telah dipilih dan dibahagi secara rawak dengan menggunakan teknik rawak sampul surat kepada 2 kumpulan iaitu kumpulan laser tanpa madu dan kumpulan laser dan madu tualang. Pesakit dinilai LogMAR BCVA dan CMT sebelum dan 3 bulan selepas rawatan. LogMAR BCVA dinilai dengan menggunakan carta LogMAR manakala CMT dinilai dengan menggunakan mesin OCT Heidelberg Spectralis.

Keputusan

Seramai 52 orang pesakit (kumpulan laser tanpa madu: 26 orang pesakit dan kumpulan laser dan madu: 26 orang pesakit) telah dipilih untuk kajian ini. Selepas 3 bulan rawatan dibuat, didapati peningkatan penglihatan purata LogMAR BCVA yang ketara pada pesakit diabetik makular edema yang dirawat dengan laser sahaja ($p= 0.002$). Walau bagaimanapun, didapati tiada perbezaan yang ketara purata LogMAR BCVA pada pesakit dirawat dengan laser antara mereka dengan dan tanpa madu pada 3 bulan selepas rawatan ($p = 0.448$). Tiada perbezaan ketara penurunan ketebalan makula direkodkan bagi nilai purata CMT ($p = 0.881$) pada 3 bulan selepas rawatan laser antara mereka dengan dan tanpa madu. Tiada pesakit yang mengalami kesan sampingan dari pemakanan madu tualang.

Kesimpulan

Pemakanan madu tualang sebagai terapi tambahan kepada rawatan laser fokal/grid menunjukkan tiada kelebihan peningkatan dari segi tahap ketajaman penglihatan dan penurunan ketebalan makula bila dibandingkan dengan rawatan monoterapi laser fokal/grid.

ABSTRACT

Introduction

Diabetic macular oedema (DMO) is a significant cause of vison loss in the diabetic patients. Laser is the standard treatment for DMO. Chronic inflammatory also has an important role in the pathogenesis of DMO. Tualang honey with anti-inflammatory and anti-oxidant properties has a potential as an adjunct treatment for DMO.

Objective

To compare LogMAR best corrected visual acuity (BCVA) and central macular thickness (CMT) at 3 months post laser treatment between those with and without honey supplement in DMO.

Methods

A prospective randomized controlled study was conducted in Hospital Universiti Sains Malaysia between April 2013 and August 2015. Patient with clinically significant macular oedema (CSMO) was selected and was randomized by using randomised envelope technique into 2 groups; laser without honey group and laser with honey group. Patients were evaluated for LogMAR BCVA and CMT pre treatment and at 3 months post treatment. LogMAR BCVA was assessed using LogMAR chart and CMT was measured using Heidelberg Spectralis OCT.

Results

A total of 52 patients were recruited (laser group without honey: 26 patients and laser with honey group: 26 patients) into this study. There was a significant improvement of mean LogMAR BCVA at 3 months post treatment in DMO patients treated with laser alone ($p=0.002$). However, there was no significant difference of the mean LogMAR BCVA in DMO patients treated with laser between those with and without honey supplement at 3 months post treatment ($p=0.448$). There was also no significant difference of mean CMT ($p=0.881$) at 3 months post laser treatment between those with and without honey supplement. There was no side effect of honey noted in patients consumed tualang honey.

Conclusion

Tualang honey used as adjunct with standard focal/grid laser has no additional improvement in both visual acuity and CMT compared to laser alone.

Chapter 1

Introduction

1.1 INTRODUCTION

Diabetic macular oedema (DMO), which can occur at any stage of diabetic retinopathy, is the main cause of vision loss in diabetic patient. A higher incidence of DMO is reported in older patients with type 2 DM (Bhagat *et al.*, 2009).

The standard treatment of DMO is laser photocoagulation proposed by Early Treatment Diabetic Retinopathy Study (ETDRS). In current approach for the treatment of DMO, intravitreal steroid and intravitreal anti vascular endothelial growth factor (VEGF) are other treatment modalities in clinical practice for DMO patients who do not respond adequately or who are refractory to laser photocoagulation. These therapeutic agents targeted at inflammatory mediators in which chronic inflammation is thought to be involved in the pathogenesis of DMO (Adamis and Berman, 2008; Antonetti *et al.*, 2006).

Several potential anti-inflammatory agent and anti-oxidant had been studied for the treatment of diabetic microvascular complication including retinopathy, nephropathy (Kowluru and Kennedy, 2001) and neuropathy (Haak *et al.*, 2000).

Honey is being recognized to have hypoglycemic effect when combined with conventional antidiabetic drug (Al-Waili, 2004; Erejuwa, 2012). Honey contains both anti-oxidants and anti-inflammatory properties. Thus, honey with its diversity of anti-oxidants has a potential as adjunct treatment to DMO patients. The aim of this study is to evaluate the effect of honey supplementation post focal laser in DMO.

1.2 BACKGROUND

1.2.1 Diabetic Macular Oedema

Diabetes mellitus (DM) is a chronic metabolic disorder with the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al.*, 2004). There is a rising prevalence of diabetes in Asian region with estimated 100 million people in China and 80 million in India (Shaw *et al.*, 2010; Yang *et al.*, 2010). According to the International Diabetes Federation, there were 3.2 millon of DM cases in Malaysia in 2014 and the prevalence of diabetes in adults in Malaysia was 16.6% (IDF, 2014). The rising prevalence of diabetes is due to change of lifestyle and aging population. In other studies, there was a significant difference in the prevalence of diabetes between Chinese (11.5%), Malay (17.1%) and Indian (21.6%) in Singapore (Chiang PP, 2011).

The prevalence of diabetic retinopathy increases with duration of diabetes. Nearly all type 1 DM and more than 60% of types 2 DM have retinopathy after 20 years (Klein *et al.*, 1998). Over 10 years, macular oedema will develop in 10% of patient with known diabetes (Ferris and Patz, 1984). In persons with diabetes, the overall prevalence of DMO was 5.7% (Wong *et al.*, 2008). The prevalence of sight threatening diabetic retinopathy in Malaysia reported by the National Eye Database (NED) in year 2007 and 2008 was 15.6% and 11.5%, respectively (Goh, 2008).

DMO is the most common cause of long term visual loss in diabetic retinopathy (ETDRS, 1985). DMO occurs as a result of exudation of fluid, lipoprotein and other plasma constituent from increased vascular permeability of microaneurysm and defective small vessel.

At initial stage of DMO, the cellular distortion is reversible. However, chronic leakages from extensive hard exudate deposition or cystoid degeneration in fovea lead to permanent loss of vision.

DMO can be classified into focal or diffuse oedema. Focal oedema is a well-circumscribed retinal leakage from microaneurysm associated with complete or incomplete perifoveal hard exudates. On the other hand, diffuse oedema is a diffuse retinal thickening with severe oedema caused by leakage from capillary dilation.

Clinically significant macular oedema (CSMO) is a form of DMO which is defined as the presence of one or more of the following features (ETDRS, 1987):

- Retinal thickening at or within 500 μm of center of the macula (Figure 1.1)
- Exudates at or within 500 μm of center of the macula with adjacent retinal thickening (Figure 1.2)
- A zone or zones of retinal thickening of one disc area or larger, any part of which is within one disc diameter from the center of the macula (Figure 1.3)

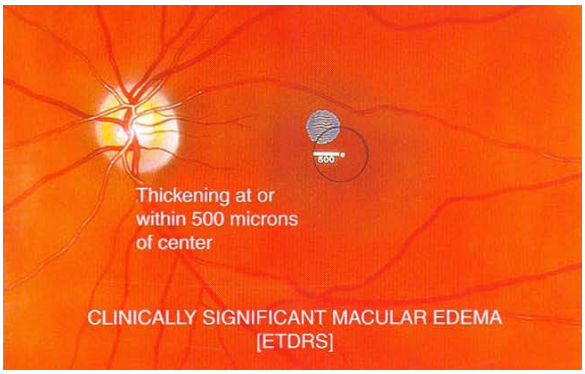

Figure 1.1: CSMO. Retinal thickening at or within 500 μm of center of the macula

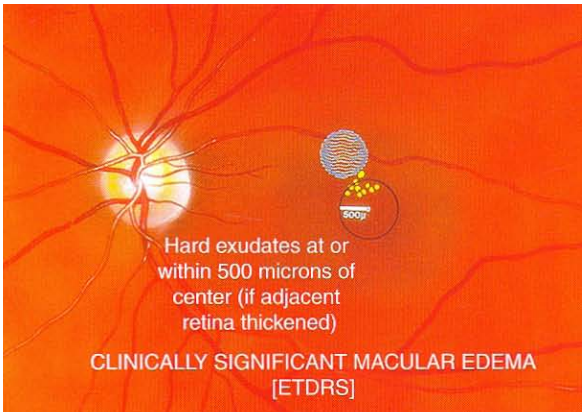

Figure 1.2: CSMO. Exudates at or within 500 μm of center of the macula with adjacent retinal thickening

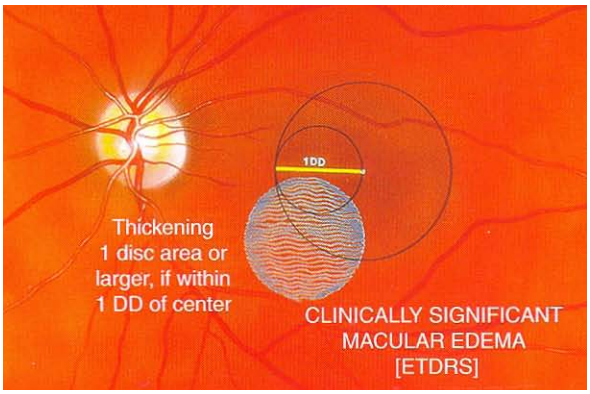

Figure 1.3: CSMO. A zone or zones of retinal thickening of one disc area or larger, any part of which is within one disc diameter from the center of macula

1.2.2 Pathophysiology of DMO

The pathogenesis of DMO is complex and involves multiple factors. It occurs mainly as a result of disruption of the blood retinal barrier, which leads to increased accumulation of fluid within the intraretinal layers of the macula. Vasoactive factors such as VEGF, protein kinase C, histamine and other factors that are related to hypoxia and chronic hyperglycemia may cause breakdown of the vitreoretinal barrier. The abnormalities in the structure of the vitreoretinal interface may also play an important role in the pathogenesis of DMO. DMO may exacerbated in the presence of vitreomacular traction (Bhagat *et al.*, 2009).

Inflammations play an important role in molecular mechanism in the pathogenesis of diabetic retinopathy. Retinal inflammation starts very early and within one week of experimental diabetic, was noted that the accumulation of leucocyte in the retinal vasculature (Adamis, 2002).

Hyperglycemia induced diabetic retinopathy is related to 4 main biochemical alterations;

i. increased polyol pathway flux
ii. increased advanced glycation end product (AGE) formation
iii. activation of protein kinase C isoforms
iv. increased hexosamine pathway flux

These pathways lead to increased oxidative stress, inflammation; vascular dysfunction resulting in increased permeability, vascular occlusion and local ischaemia. Upregulation of pro-angiogenic and pro-inflammatory factors such as VEGF, insulin like growth factor (IGF), angiopoetins-2 (Ang-2), stromal derived factor-1 (SDF-1),

basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), tumour necrosis factor (TNF) and interleukin-6. Adhesion molecules also play a role in inflammation, blood vessel endothelial cells characterictically respond to pro-inflammatory stimuli and recruit leukocytes by selectively expressing adhesion molecules on the surface, such as vascular cell adhesion molecules-1 (VCAM-1), intercellular adhesion molecules-1 (ICAM-1) and endothelial cell-selectin (E-selectin) (Tunon *et al.*, 2009).

The postulated inflammatory events involved in the endothelial barrier alteration in DMO include:

i. Increased expression of endothelial adhesion molecules such as ICAM, VCAM, platelet endothelial cell adhesion molecule (PECAM) and platelet-selectin (P-Selectin) (Adamis and Berman, 2008; McLeod *et al.*, 1995)
ii. Adhesion of leucocytes to the endothelium (Miyamoto *et al.*, 1999)
iii. Release of inflammatory cytokines, vascular permeability factors and growth cytokines (Yuuki *et al.*, 2001)
iv. Alteration of adherens and tight junctional proteins such as vascular endothelial-cadherin (VE-Cadherin) and zona occludens-1 (ZO-1), Claudin (Harhaj *et al.*, 2006; Murakami *et al.*, 2009)
v. Infiltration of leucocytes (diapedesis) into retina resulting in the alteration of the blood retinal barrier (Muller, 2003)

Increased leucocyte adhesion results in loss of endothelial cells and breakdown of the blood retinal barrier (Joussen *et al.*, 2001).

Oxidative stress is increased in diabetic retinopathy (Naziroglu and Butterworth, 2005). The possible causes include increased production of free radicals or impaired anti-oxidant defense system in the diabetic retina (Kowluru *et al.*, 2003).

Retina is susceptible to oxidative stress because of its high consumption of oxygen and high proportion of polyunsaturated fatty acids (Anderson *et al.*, 1984). Increased oxidative stress is associated with increase in retinal basement thickening (Robison *et al.*, 2000) and pericyte dropout in diabetic retinopathy (Li *et al.*, 1999). In diabetic retina, production of retinal free radicals exceeds their removal; anti-oxidant defence systems such as catalase and glutathione peroxidase are subnormal (Kowluru *et al.*, 1997). Increased reactive oxygen species can activate nitric oxide synthase resulting increased nitric oxide (Ho *et al.*, 1999; Miralles *et al.*, 2000). Elevated levels of nitric oxide are seen remained in diabetic retina for 12-14 months resulted pericyte loss, acellular capillaries and basement membrane thickening (Kowluru *et al.*, 2001).

Retinal nitric oxide has been postulated in ischaemic injury and inhibition of nitric oxide synthase can protect the retina from ischaemic damage (Roth, 1997).

1.2.3 Investigation of DMO

1.2.3.1 Optical Cohence Tomography

Optical Cohence Tomography (OCT) is a diagnostic imaging technique that produces micrometer resolution, cross sectional images of posterior segment of the eye. It is a non-invasive and non-contact imaging modality that provides high resolution quantitative measurement of macular thickness.

OCT is based on the principle of low coherence optical interferometry, using near infra red light (800-850 nm wavelength) directed to retina and produce backscattered light from different layers in the retina. A two dimensional image is constructed and displayed in grey or colour scales. White represents areas of high reflectivity whereas black represents areas of low reflectivity.

There are 2 types of OCT, Time Domain OCT (TD-OCT) and Spectral Domain OCT (SD-OCT). TD-OCT can only obtain 400 A scans per second whereas SD-OCT can acquire greater detail over 20,000 A scans per second. SD-OCT scans in a shorter time, 0.072 seconds whereas the TD-OCT scans within 1.23 seconds. TD-OCT scans 6 x 6 mm centered on the fovea. On the other hand, SD-OCT can image the entire macula at 2-10 μm in axial (depth) resolution and 20 μm in horizontal resolution. Due to faster scanning speed, SD-OCT improved accuracy of retinal thickness and volumes measurement. It also offers improving registration; so serial imaging is now possible.

OCT also produce a retinal thickness map with computer software, which can automatically detect inner and outer retinal boundaries and produces a macular topographic map.

In DMO, OCT can be used to help and quantify the amount of oedema present (Figure 1.4), the proximity and involvement of the foveal region by the oedema as well as the response to therapy (Figure 1.5).

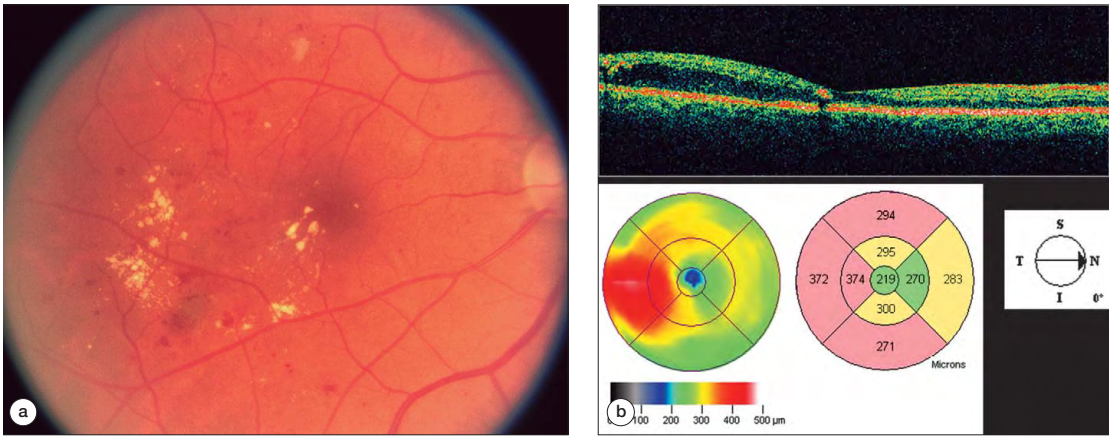

Figure 1.4: OCT and corresponding fundus image in a patient with DMO (Albert *et al.*, 2008)

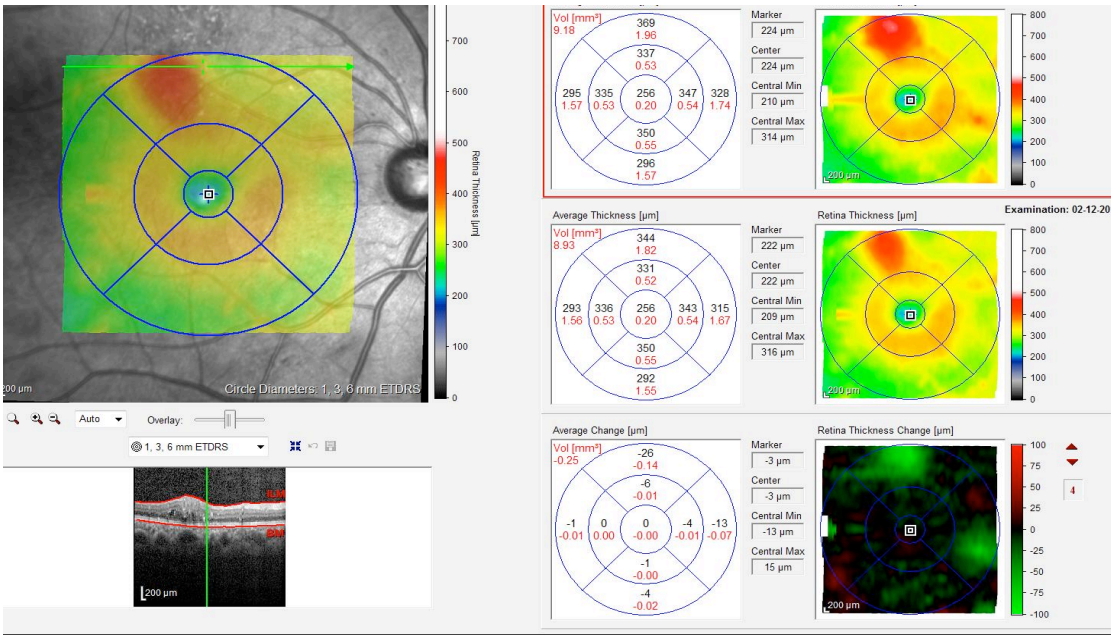

Figure 1.5: Retinal thickness map derived from Spectralis OCT. The thickness for each nine macular subfields is displayed in both numerical and colour coded formats.

1.2.3.2 Fundus Fluorescein Angiography

Fundus Fluorescein Angiography (FFA) is a method of investigation to detect disruption of the blood retinal barrier in order to determine the presence and the extent of macular oedema. It is a method in which fluorescein sodium is administered intravenously followed by rapid sequence photography of the retina to evaluate its circulation.

Normally, the choroidal filling is seen due to fenestrated choriocapillaris allow free passing of fluorescein sodium whereas inner blood retinal barrier does not allow flourescein leakage. Flourescein can leak out of retinal capillaries into the retina only when the capillary endothelium is damaged as in diabetic retinopathy. FFA provides enhanced visualization of the geometry and distribution of macular oedema.

1.2.4 Treatment Options For Diabetic Macular Oedema

1.2.4.1 Laser Photocoagulation

Laser photocoagulation, focal or grid is the standard treatment for DMO. The ETDRS group (1987) demonstrated that eyes with macular oedema benefited from immediate focal argon laser photocoagulation, which significantly reduces the risk of moderate visual loss. Focal laser most beneficial for eyes meeting criteria for CSMO in which incidence of visual loss is decreased by 50% at 3 years (ETDRS, 1985).

The mechanism of DMO resolution after laser therapy is unknown. The possible mechanisms include:

- Laser-induced destruction of oxygen-consuming photoreceptores. Oxygen which normally diffuses from the choriocapillaris into outer retina can diffuse through the laser scar to the inner retina, relieving inner retinal hypoxia.
- Laser photocoagulation induced retinal pigmented epithelium proliferation that produce cytokines to inhibit the VEGF effect.

There is no major adverse effect with laser theraphy except minor loss of visual field and occasionally paracentral scotoma.

1.2.4.2. Intravitreal Steroids

Steroids may be useful to treat DMO because of anti-inflammatory effects which include decreased inflammatory cell activation. Steroids also have a direct effect on endothelial cell junctions maturation and improved blood retinal barrier properties (Albert *et al.*, 2008).

Diabetic Retinopathy Clinical Research Network (DRCR.net, 2008) carried out a multicenter randomised controlled trial comparing laser photocoagulation with either 1 mg or 4 mg of intravitreal triamcinolone. In first year, mean visual acuity was better in intravitreal triamcinolone (IVTA) groups than in laser group. However, after 2 years of treatment, it significantly better in the laser group than in the IVTA groups. The main side effect of intravitreal steroid are the development of cataract and elevation of intraocular pressure (Gillies *et al.*, 2004).

1.2.4.3 Intravitreal VEGF Inhibitors

VEGF-A is a major mediator of increased retinal permeability. Blockage of VEGF has shown to reduce vascular permeability (Hippenstiel *et al.*, 1998).

Many trials and studies have demonstrated that inhibition of VEGF such as pegaptanib, bevacizuman and ranibizumab has shown dramatic improvements in resolution of DMO and improvement in visual acuity.

The Ranibizumab for Edema of the Macula in Diabetes (READ-2 study, 2010) compared the effect of intravitreal 0.5 mg ranibizumab versus laser photocogualation versus combined ranibizumab and laser photocoagulation. This study demonstrated

that the mean gain in best corrected visual acuity (BCVA) was significantly better in the ranibizumab group compared to the laser photocoagulation group. There was no significant difference between the ranibizumab group and the combination group (Nguyen *et al.*, 2010).

1.2.4.4. Vitrectomy

It has been postulated that the role of the vitreous in DMO which involves mechanical traction of the vitreoretinal interface and the accumulation of factors altering vascular permeability such as VEGF in the vitreous (Bhagat *et al.*, 2009).

Vitrectomy with posterior hyaloid removal or internal limiting membrane peeling can remove these factors in DMO. The DRCR.net (2010) conducted a study to evaluate vitrectomy for DMO with at least moderate vision loss and vitreomacular traction. The study reported that at 6 months, 68% of the vitrectomized eyes had at least a 50% reduction in central retinal thickness. Visual acuity improved by 10 letters or more in 38% of vitrectomized eyes and deteriorated by 10 letters or more in 22% of vitrectomized eyes. The complications of vitectomy reported in this study were vitreous haemorrhage, elevated intraocular pressure, retinal detachment and endophthalmitis.

1.2.5 Honey

1.2.5.1 Component of Honey

Honey is a natural sweetener. Honey is produced from many different floral sources and its biochemical and pharmacological activities vary depending on its origin and processing. Tualang honey is produced by the bees *Apis dorsata*, which build hives on tualang tree.

The composition of honey per 100 g is 300 calories, water (17.1 g), total carbohydrates (82.4 g), frustose (38.5 g), glucose (31 g), maltose (7.2 g), sucrose (1.5 g), total proteins (0.3 g), total fat and cholesterol (0), ph 3.4-6.1 and water content (16%-18.3%). Honey contains a variety of biologically active compounds such as flavonoids, vitamins and anti-oxidants (Chua et al., 2013). Honey also contains various enzymes such as oxidase, invertase, amylase and catalase.

1.2.5.2 Anti-oxidant Effects of Honey

Honey contains both aqueous and lipophillic anti-oxidants. Honey with higher water content and with darker colour has more anti-oxidants. The anti-oxidants composition in honey include monophenolics, flavonoids, polyphenolics and Vitamin C (Schramm *et al.*, 2003). This study also had shown that there was a significant increase of plasma total phenolic and plasma anti-oxidant following honey consumption (Schramm *et al.*, 2003). Study done by Al-Mamary et al, (2002) had reported on composition of honeys and their biological properties as anti-oxidant. Honey can increase total anti-oxidant status, activities of glutathione S-transferase and glutathione reductase in diabetic rats (Erejuwa *et al.*, 2010). In that animal study also shown

reduction of lipid peroxidation and improvement of renal morphology in honey treated diabetic rats (Erejuwa *et al.*, 2010). Honey can reduce serum fructosamine in diabetic that may attributed to anti-oxidant effect (Erejuwa OO, 2011). Fructosamine can form AGE which implicated diabetic complications (Selvaraj *et al.*, 2006).

Honey also contains radical scavenger which are able to reduce the imbalance between free radical production and anti-oxidant level (Kishore *et al.*, 2011).

With regard to diabetic retinopathy, animal study had shown that combination of multiple types of anti-oxidants significantly provide more protection than single anti-oxidant supplements (Kowluru *et al.*, 2001). Thus, honey with its diversity of anti-oxidants has a potential as an adjunct treatment for DMO.

1.2.5.3 Anti-Inflammatory Effects of Honey

Honey is also known to have anti-inflammatory properties. A study reported the efficacy of honey in the healing of cutaneous wounds of rabbits on the basis of histopathological and biochemical changes (Oryan and Zaker, 1998). The honey treated rabbits showed less oedema and necrosis, fewer polymorphonuclear cell infiltration, better wound contraction and improved epithelialization. A recent study observed that honey has anti-inflammatory components that reduced corneal infiltration by inflammatory cell in alkali chemical eye injury of rabbits (Bashkaran *et al.*, 2011). Honey administered to rats with inflammatory bowel disease showed significantly reduced myeloperoxidase (Bilsel *et al.*, 2002).

A study that investigated the effects of honey in animal studies reported that honey reduced oedema and pain in the inflammatory tissues. The reduced oedema and pain corelates with the inhibition of nitric oxide and prostaglandin E(2) (Kassim *et al.*, 2010; Owoyele *et al.*, 2011).

1.3 RATIONALE OF STUDY

DMO is a vision threatening complication in DM. Primary standard care of treatment for DMO is laser photocoagulation (ETDRS, 1987).

Honey is being recognised to have hypoglycemic effect when combined with conventional antidiabetic drug (Al-Waili, 2004; Erejuwa, 2012). Tualang honey was reported to reduce hyperglycemia and ameliorates oxidative stress in kidney of diabetic rats (Erejuwa et al., 2011).

Since the honey has anti-inflammatory and anti-oxidant properties, therefore it has potential therapeutic agent for treatment of DMO. Flavonoid has been investigated and showed to reduce inflammmatory factors in diabetic retinopathy (Kowluru et al., 2014).

Chapter 2

Objective

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To evaluate the visual acuity and macular thickness post focal laser with and without supplementary honey in DMO

2.2 SPECIFIC OBJECTIVE

2.2.1 To compare LogMAR BCVA post focal laser (pre and post) between those with and without supplementary honey in DMO

2.2.2 To compare central macular thickness (CMT) post focal laser (pre and post) between those with and without supplementary honey in DMO

Chapter 3

Methodology

3. METHODOLOGY

3.1 STUDY DESIGN

Prospective randomised control study

3.2 POPULATION, PLACE AND PERIOD OF STUDY

Study population: All Type 2 DM patients diagnosed with CSMO presented to Ophthalmology Clinic and Diabetic Center, Hospital Universiti Sains Malaysia (USM), Kelantan

Place of study: Ophthalmology Clinic and Diabetic Center, Hospital USM, Kelantan

Period of study: April 2013 to August 2015

3.3 ETHICAL BOARD APPROVAL

This study received approval from the Research and Ethical Committee, School of Medical Sciences, USM on 27th March 2013 (Appendix A).

3.4 FINANCIAL SUPPORT

This study was partially funded by Research University Team grant (1001/PPSP/852001) from USM.

3.5 SELECTION CRITERIA

3.5.1 Inclusion Criteria

i. Type 2 DM patient with CSMO
ii. Age between 30 to 75 years old
iii. Clear media to be able to perform acceptable OCT for macular thickness measurement
iv. HbA1c less than 10 %

3.5.2 Exclusion Criteria

i. CSMO associated with severe non proliferative diabetic retinopathy or proliferative diabetic retinopathy
ii. History of previous laser photocoagulation
iii. History of intravitreal injection (e.g. steroid, anti-VEGF)
iv. History of intraocular surgery (e.g. within 6 months post-cataract operation, vitreous surgery, retinal detachment surgery)
v. Patient with any other ocular disease such as glaucoma or pre-existing macular disorder such as age related macular degeneration
vi. History of taking anti-inflammatory agents
vii. History of taking oral natural herbal health products / dietary supplements within 1 month prior to study

3.6 SAMPLE SIZE

Sample size calculation was done using PS software – Power and Sample Size calculation version 3.0.43 using the t-test formula.

α : level of significance

SD : standard deviation

DD : detected difference

n : sample size

Objective 1: To compare LogMAR BCVA post focal laser (pre and post) between those with and without supplementary honey in DMO

α : **0.05**

Power : **0.8**

DD : **LogMAR -0.12 (**the smallest, clinically meaningful difference in mean visual acuity (pre and post) between those with and without supplementary honey in DMO patients that is desired to be detected)

SD of the change in the LogMAR BCVA (pre and post) in diabetic macular oedema : LogMAR 0.18 (Faghihi *et al*, 2008)

n : 20 (for each group)

20 + 10% dropout = **22 patients**

Objective 2: To compare CMT post focal laser (pre and post) between those with and without supplementary honey in DMO

α : **0.05**

Power : **0.8**

DD : **48 um (**the smallest, clinically meaningful difference in mean macular thickness between those with and without supplementary honey in DMO patients that is desired to be detected)

SD of the change in the CMT (pre and post) in DMO patients : **88 um** (Bandello et al., 2005)

n : 28 (for each group)

28 + 10% dropout = 31 **patients**

Sample size chosen for this study: **31 patients**

3.7 SAMPLING METHOD

All Type 2 DM patients diagnosed with CSMO attending Ophthalmology Clinic Hospital USM between April 2013 and August 2015 that fulfill the criterias were recruited into the study.

3.8 RANDOMISATION

New cases of CSMO who fulfilled the selection criterias and given consent for this study were randomised into 2 groups (Laser without Honey Group and Laser with Honey Group) using randomised opaque envelope technique.

The envelope was prepared with half of the envelopes containing a piece of paper with the word ‘Laser without Honey’ and the remaining half stated ‘Laser with Honey’. These envelopes were shuffled and kept in a randomisation room in Hospital USM. The envelope was drawn for each patient by co-investigator A (CAB).

3.9 DEFINITION OF TERMS

3.9.1 Diabetic Macular Oedema

DMO is retinal thickening with or without deposits of intraretinal hard exudates. CSMO classification according to EDTRS (1987) is the definition of DMO that was used in this study. CSMO is defined as the presence of one or more of the following features:

- Retinal thickening at or within 500 μm of the center of the macula
- Exudates at or within 500 μm of the center of the macula with adjacent retinal thickening
- A zone or zones of retinal of thickening of one disc area or larger, any part of which is within one disc diameter from the center of the macula

3.9.2 Focal Laser Photocoagulation

Focal laser photocoagulation is the standard treatment for DMO. Argon green laser applied to all leaking microaneurysms between 500 and 3000 μm (2 disc diameter from center of the macula) to cause regression of macular oedema and to prevent progressive macular oedema in the future. Laser machine Visular 532S (Carl Zeis) was used in this study.

3.9.3 Macular Thickness

Macular thickness is the volumetric measurement in three dimensions using OCT. CMT is the central 1 mm area of a 6 mm mapping as described in ETDRS study (ETDRS, 1985) as shown in Figure 3.1. The CMT was taken for analysis in this study. OCT Heidelberg Spectralis was used in this study for CMT measurement.

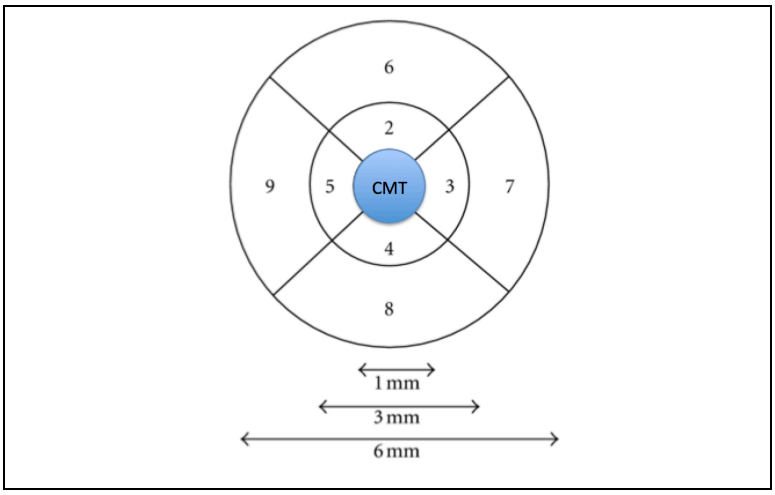

Figure 3.1: Central macular thickness (CMT) area of 1 mm (ETDRS, 1985)