CHANGES OF CENTRAL MACULAR THICKNESS POST INTRAVITREAL RANIBIZUMAB AND ITS ASSOCIATED FACTORS AMONG DIABETIC MACULAR OEDEMA PATIENTS

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TABLE OF CONTENT

ACKNOWLEDGEMENTS ..... **ii**
TABLE OF CONTENT ..... **iii**
LIST OF TABLES ..... **vii**
LIST OF FIGURES ..... **viii**
LIST OF ABBREVIATIONS ..... **ix**
LIST OF SYMBOLS ..... **x**
ABSTRAK ..... **xi**
ABSTRACT ..... **xiii**

CHAPTER 1 INTRODUCTION **1**
1.1 Diabetic Mellitus and Its Complications 1
1.2 Diabetic Macular Oedema and Its Treatment 3
1.3 Problem Statement 6
1.4 Justifications and Benefit of the study 6
1.5 Research Questions 8
1.6 Research Objectives 8
1.6.1 General Objectives 8
1.6.2 Specific Objectives 8
1.7 Research Hypotheses 8

CHAPTER 2 LITERATURE REVIEW **9**
2.1 Introduction 9
2.2 Search Terms and Databases 9
2.3 Central Macular Thickness 11
2.4 Diabetic Macular Oedema 12
2.4.1 Clinical Feature of Diabetic Macular Oedema 13
2.4.2 Pathophysiology of Diabetic Macular Oedema 14
2.4.3 Risk Factors of Diabetic Macular Oedema 16
2.4.4 Prevention of Diabetic Macular Oedema 17
2.4.5 Treatment Options of Diabetic Macular Oedema 18
2.5 Anti-Vascular Endothelial Growth Factor Drugs 19
2.6 Efficacy of Intravitreal Ranibizumab 20
2.7 Factors Associated with Changes of Central Macular Thickness 22

2.7.1 Systemic Factors 23
2.7.1.1 Age 23
2.7.1.2 Sex 23
2.7.1.3 Ethnicity 24
2.7.1.4 Duration of Diabetes Mellitus 24
2.7.1.5 Cardiovascular Disease 25
2.7.1.6 HbA1c 25
2.7.1.7 Antihyperglycemic Medication 26
2.7.2 Ocular Factors 26
2.7.2.1 Severity of Diabetic Retinopathy 26
2.7.2.2 Baseline Parameters 27
2.7.2.3 Eye Features 28
2.8 Conceptual Framework 29

CHAPTER 3 METHODOLOGY 30
3.1 Study Design 30
3.2 Study Location 30
3.3 Study Duration 30
3.4 Study Population and Sample 30
3.4.1 Reference Population 30
3.4.2 Source Population 31
3.4.3 Sampling Frame 31
3.4.3.1 Inclusion Criteria 31
3.4.3.2 Exclusion Criteria 31
3.4.4 Sample Size Determination 32
3.4.5 Sampling Method and Subject Recruitment 34
3.5 Research Tool 34
3.6 Data Collection 35
3.7 Data Variables 36
3.8 Operational Definition 37
3.9 Statistical Analysis 38
3.9.1 Data Exploration and Cleaning 38
3.9.2 Descriptive Statistics 38
3.9.3 Univariable Analysis (Simple Linear Regression) 39
3.9.4 Multivariable Analysis (General Linear Regression) 39
3.9.5 Checking for Multicollinearity and Interaction 40

3.9.6 Checking for Assumption 41
3.9.6.1 Random Sampling 41
3.9.6.2 Independent Observation 42
3.9.6.3 Linearity or Overall Model Fitness 42
3.9.6.4 Equal Variance 43
3.9.6.5 Normality of Residual 43
3.9.6.6 The Linearity of Each Independent Variable 43
3.9.7 Regression Diagnostics 44
3.9.8 Remedial Measure 45
3.9.9 Data Presentation, Interpretation and Conclusion 46
3.9.10 Summary of Statistical Analysis 47
3.10 Study Flow Chart 48
3.11 Ethical Consideration and Confidentiality 49

CHAPTER 4 RESULTS 50
4.1 Number of Patients Included 50
4.2 Baseline Characteristics 50
4.2.1 Socio-demographic Characteristics 50
4.2.2 Disease-related Characteristics 51
4.2.3 Comorbidities Characteristics 53
4.2.4 Ocular-related Characteristics 54
4.3 Central Macular Thickness Measurement 54
4.4 Treatment Response 55
4.5 Univariable Analysis 55
4.6 Variable Selection (General Linear Regression) 58
4.7 Checking of Interaction and Multicollinearity 59
4.8 Regression Model Assumption 60
4.8.1 Independent Samples 60
4.8.2 Interaction between Each Independent Variable 60
4.8.3 Overall Linearity and Equal Variance 61
4.8.4 Normality of Residuals 62
4.8.5 Linear Relationship between Residuals and Numerical Independent Variable 63
4.9 Regression Diagnostics 64
4.9.1 Regression Diagnostic for Outliers 64
4.9.2 Regression Diagnostic for Influential Cases 65

4.10 Remedial Measures 68
4.11 Final Model of the Study 69
4.12 Interpretation of the Results 71

CHAPTER 5 DISCUSSION 72
5.1 Profile of Study Subjects 72
5.2 Changes of Central Macular Thickness 76
5.3 Treatment Response 78
5.4 Factors Associated with Changes of Central Macular Thickness 79
5.4.1 Baseline Central Macular Thickness 79
5.4.2 Presence of Subretinal Fluid 80
5.4.3 Other Factors 82
5.5 Statistical Analysis 86
5.6 Strength of the Study 88
5.7 Limitations of the Study 89

CHAPTER 6 CONCLUSION AND RECOMMENDATIONS 91
6.1 Conclusion 91
6.2 Recommendations 92

REFERENCES 94
APPENDIX A: DATA EXTRACTION FORM
APPENDIX B: ETHICAL APPROVAL
APPENDIX C: APPROVAL LETTER FROM THE DIRECTOR OF HOSPITAL USM
APPENDIX D: DATA ANALYSIS SYNTAX
LIST OF PUBLICATIONS

LIST OF TABLES

Table 2.1:	Results of search term in respective database	10
Table 3.1:	List of clinically important variables selected for multivariable analysis	36
Table 4.1:	Descriptive statistics for socio-demographic characteristics of DMO patients on IVR treatment in Hospital USM (n=153)	51
Table 4.2:	Descriptive statistics for disease-related characteristics of DMO patients on IVR treatment in Hospital USM (n=153)	52
Table 4.3:	Descriptive statistics for comorbidities of DMO patients on IVR treatment in Hospital USM (n=153)	53
Table 4.4:	Descriptive statistics for ocular-related factors of DMO patients on IVR treatment in Hospital USM (n=153)	54
Table 4.5:	The estimation of mean and 95% CI for the CMT measurements (n=153)	54
Table 4.6:	Proportion of treatment response after three months of injections of IVR (n=153)	55
Table 4.7:	Associated factors of changes of CMT at month three after IVR treatment by Simple Linear Regression (n=153)	56
Table 4.8:	Variable selected and included in the preliminary main effect model by automated variable selection method (n=153)	58
Table 4.9:	Variance Inflation Factor (VIF) and tolerance values for each variable	59
Table 4.10:	Changes in the regression coefficients of the variables in the preliminary model with and without potential outliers	68
Table 4.11:	Changes in the significance of the variable in the preliminary model with and without potential outliers	69
Table 4.12:	Factor associated with changes of CMT in DMO patients receiving IVR (n = 153)	70

LIST OF FIGURES

Figure 2.1:	Basic parts of the human eye	11
Figure 2.2:	Macular thickness map by OCT	12
Figure 2.3:	DMO A: Mild; B: Moderate; C: Severe	13
Figure 2.4:	Cross-sectional retinal layer imaged by OCT	14
Figure 2.5:	Evaluation of macular thickness by OCT	16
Figure 2.6:	The relationships between associated factors and treatment effect on CMT	29
Figure 3.1:	Flowchart summary of statistical analysis using general linear regression	47
Figure 3.2:	The flow chart of the study	48
Figure 4.1:	Scatter plot of residuals against the predicted value of changes of CMT	61
Figure 4.2:	Histogram of residual values of changes of CMT	62
Figure 4.3:	Scatter plot of residuals values of changes of CMT against baseline CMT	63
Figure 4.4:	Scatter plot of Studentised residuals against the predicted value (cut-off point y line =3 | -3)	64
Figure 4.5:	Scatter plot of Leverage against the predicted value (cut-off point y line =0.04)	65
Figure 4.6:	Scatter plot of Cook's Distance statistics against the predicted value (cut-off point y line =0.5)	66
Figure 4.7:	Scatter plot of DFITS against the predicted value (cut-off point y line =1)	66
Figure 4.8:	Scatter plot of DFBETA for baseline CMT variable against the predicted value (cut-off point y line =1)	67
Figure 4.9:	Scatter plot of DFBETA for the presence of SRF variable against the predicted value (cut-off point y line =1)	67

LIST OF ABBREVIATIONS

CI	Confidence interval
CMT	Central macular thickness
DM	Diabetes mellitus
DMO	Diabetic Macular Oedema
DR	Diabetic retinopathy
ELM	External limiting membrane
EMA	European Medicines Agency
ERM	Epiretinal membrane
FDA	Food and Drug Administration
GCC	Ganglion cell complex
IQR	Interquartile range
IVR	Intravitreal Ranibizumab
NPDR	Non-Proliferative Diabetic Retinopathy
OCT	Optical Coherence Tomography
PDR	Proliferative Diabetic Retinopathy
PRP	Pan Retinal Photocoagulation
RPE	Retinal pigment epithelium
SD	Standard Deviation
SDR	Studentized deleted residuals
SE	Standard error
SRF	Subretinal fluid
VA	Visual acuity
VEGF	Vascular endothelial growth factor
VIF	Variance inflation factor

LIST OF SYMBOLS

α	Alpha/ level of significance
n	Number of samples
f2	Cohen’s effect size
b	Regression coefficient
1-β	Power of the study
≤	Less than or equal
>	More than
%	Percentage

ABSTRAK

PERUBAHAN KETEBALAN MAKULA TENGAH SELEPAS RANIBIZUMAB INTRAVITREUS DAN FAKTOR-FAKTOR YANG BERKAITAN DALAM KALANGAN PESAKIT DIABETIS EDEMA MAKULA

Latar Belakang: Ranibizumab intravitreus (IVR) ialah *anti-vascular endothelial growth factor (anti-VEGF)* yang menjadi rawatan pilihan untuk memperbaiki penglihatan dalam kalangan pesakit diabetis edema makula. IVR bertindak dengan menghalang *VEGF-A* daripada mengikat pada reseptor lalu mengurangkan kebolehtelapan vaskular dan seterusnya menyebabkan perubahan pada ketebalan makula tengah (MT). Tindak balas kepada rawatan IVR dikira optimum sekiranya perubahan MT selepas tiga bulan adalah kurang atau sama dengan 280 µm. **Objektif:** Objektif kajian ini ialah untuk memperihalkan min perubahan ketebalan MT dan perkadaran tindakbalas yang optimum selepas tiga bulan menerima rawatan IVR serta mengenalpasti faktor-faktor yang berkaitan dengan ketebalan MT dalam kalangan pesakit diabetis edema makula. **Kaedah**: Kajian keratan rentas dengan menyemak 153 rekod sekunder pesakit diabetis edema makula yang telah menerima tiga bulan rawatan IVR di Hospital Universiti Sains Malaysia dari tahun 2016 sehingga 2019. Ketebalan MT diukur dengan menggunakan mesin *Optical Coherence Tomography (OCT)*. Perubahan ketebalan MT dikira berdasarkan perbezaan nilai ketebalan MT dalam µm antara sebelum dan selepas tiga bulan menerima rawatan IVR. Regresi linear umum digunakan untuk menganalisis hubungkait antara faktor-faktor tersebut dengan perubahan ketebalan MT menggunakan perisian STATA SE 14.

Keputusan: Sebanyak 153 pesakit diabetis edema makula yang terlibat. Terdapat 69 (45.1%) pesakit lelaki dan 84 (54.9%) pesakit perempuan dengan purata dan sisihan piawai bagi umur pesakit ialah 57.5 (7.70) tahun manakala median dan julat antara kuartil bagi tempoh pesakit menghidap diabetes ialah 11 (9) tahun. Purata dan sisihan piawai bagi perubahan ketebalan MT ialah 155.5 (137.8) µm. Selepas tiga bulan menerima rawatan IVR, hanya 30.7% pesakit mencapai tindakbalas yang optimum. Faktor-faktor yang signifikan berkaitan dengan perubahan ketebalan MT adalah nilai asal ketebalan MT sebelum rawatan (b =0.73; 95% selang keyakinan: 0.63-0.84; p = <0.001) dan adanya cecair subretinal (b= 35.43; 95% selang keyakinan: 3.70 - 67.16; p = 0.029). Faktor-faktor ini menjelaskan 58.3% variasi dalam perubahan ketebalan MT. **Kesimpulan:** Perubahan ketebalan MT dan kadar peratusan pesakit yang mencapai tindakbalas yang optimal adalah rendah selepas tiga bulan menerima rawatan IVR. Pesakit yang mempunyai cecair subretinal dan mempunyai nilai asal ketebalan MT yang tinggi akan mempunyai kadar perubahan ketebalan MT yang lebih tinggi selepas tiga kali menerima injeksi IVR.

Kata kunci: diabetis edema makula, *anti-VEGF*, Ranibizumab intravitreus, ketebalan makula tengah

ABSTRACT

CHANGES OF CENTRAL MACULAR THICKNESS POST INTRAVITREAL RANIBIZUMAB AND ITS ASSOCIATED FACTORS AMONG DIABETIC MACULAR OEDEMA PATIENTS

Background: Intravitreal Ranibizumab (IVR) which is an anti-vascular endothelial growth factor (anti-VEGF) has become the preferred treatment option to improve the vision of diabetic macular oedema (DMO) patients. IVR acts by inhibits VEGF-A from binding to its receptors, leading to decreased in vascular permeability and thereby causing changes of Central Macular Thickness (CMT). The response to IVR treatment is considered optimal if the changes of CMT after three months is less or equal to 280 µm. **Objectives:** The objectives of this study were to estimate the mean of changes of CMT and the proportion of optimal treatment response after three injections of IVR and to identify associated factors of changes of the CMT among DMO patients. **Methods:** This was a cross-sectional study using secondary record review of the DMO patients who received three-month treatment of IVR in Hospital Universiti Sains Malaysia from 2016 to 2019. The CMT was measured by using Optical Coherence Tomography (OCT) machines. Changes of CMT was calculated based on the differences of thickness of central macula in µm between baseline and at month three. General linear regression was then applied to analyse the association of changes of CMT using STATA SE 14 software. **Results:** A total of 153 DMO patients were included. There were 69 (45.1%) male and 84 (54.9%) female patients with a mean (standard deviation (SD)) age of 57.5 (7.70) years and median (interquartile range (IQR)) of diabetes duration of 11 (9) years. The mean (SD) of changes of CMT was 155.5 (137.8) µm. After three injection of IVR, only 30.7% had optimal treatment

response. Factors significantly associated with changes of CMT were baseline CMT (b =0.73; 95% CI: 0.63,0.84; p = <0.001) and presence of subretinal fluid (SRF) (b= 35.43;95% CI:3.70,67.16; p = 0.029). These factors explained 58.3% of the variation in changes of CMT. **Conclusions:** There was less changes of CMT and less patients achieved optimal treatment response after three months of IVR treatment. Patients who presented with SRF and high baseline CMT had greater changes of CMT after receiving three injections of IVR treatments.

Keywords: diabetic macular oedema, anti-VEGF, intravitreal ranibizumab, central macular thickness

CHAPTER 1

INTRODUCTION

1.1 Diabetic Mellitus and Its Complications

Diabetes mellitus (DM) is one of the leading public health concerns worldwide (World Health Organization, 2018b). It is defined as a chronic and metabolic disorder characterised by inadequate secretion or utilisation of insulin and in the long term may lead to several complications (World Health Organization, 2018b). There are 425 million adults with aged more than 18 years old living with diabetes worldwide, and there is a possibility that this figure will rise to 629 million in 2045 (International Diabetes Federation, 2017b). Based on the National Health and Morbidity Survey (NHMS) 2015 conducted in Malaysia, the prevalence of diabetes has risen from 15.2% in 2011 to 17.5% in 2015 for adult above 18 years old. In Kelantan, however, the survey reported that there is a slight decrease from 19.7% in 2011 to 18.5% in 2015 of the overall prevalence of diabetes (Institute for Public Health, 2015).

Patient with poor glycaemic control of diabetes has an increased risk of developing diabetes complications. There are two main types of diabetes complication which are macrovascular and microvascular complications. The macrovascular complications such as stroke and cardiovascular disease are due to damage to larger blood vessels in the brain and the heart respectively, while the microvascular complications include diabetic neuropathy, diabetic nephropathy and diabetic retinopathy (World Health Organization, 2018b). Diabetic neuropathy is due to any damage of the

nerves in the body while diabetic nephropathy and diabetic retinopathy are due to damage to small blood vessels in the kidneys and eyes, respectively.

Diabetic retinopathy (DR) is the most common microvascular complications in the eyes that affects over one-third of people with DM, and it is projected that approximately 93 million people globally have DR (International Diabetes Federation, 2017b). This complication causes damage to the blood vessels in the light-sensitive area lining at the back of the eye which is known as the retina, leading to early clinically visible appearance of microaneurysm formation and intraretinal haemorrhages (American Academy of Ophthalmology Retina/Vitreous Panel, 2014). As a result, patients may experience cloudy or blurred vision, having a dark spot in the centre of their vision as well as the difficulty to see at night. Without treatment, it may lead to vision impairment or blindness. Hence, diabetic patients must have a retinal screening regularly as this complication is preventable if detected and intervened in its early stage (Lee *et al.*, 2015).

In Malaysia, The Diabetic Eye Registry 2007 has reported that from 10,586 diabetics eyes examined, 36.8% of them had DR (Goh *et al.*, 2010) . Besides, patients who had a longer duration of DM are more prone to develop DR (American Diabetes, 2012). It was reported that patients who had DM for more than 15 years had approximately 2% chance of becoming blind and 10% chance to develop severe visual disability due to DR (International Diabetes Federation and World Health Organization, 2000). Diabetic Retinopathy is asymptomatic in its early stage, and this complication starts to develop when metabolic abnormalities such as chronically high levels of blood sugar, hypertension and vascular inflammation are causing damage and obstruction of

the retinal blood vessels (Boyer *et al.*, 2013). Over time, this microvascular damage may then progress through several stages depending on the level of disease severity. The stages include non-proliferative DR, proliferative DR and also diabetic macular oedema (DMO). The main consequence of DR includes eyesight deterioration or even blindness in diabetic patients (Zhang *et al.*, 2014). It was reported that there was 4.8% of the 37 million cases of blindness worldwide due to DR (World Health Organization, 2018a). In Malaysia, the National Eye Survey (NESII) reported that the prevalence of DR as the causes of blindness was 10.4% among the 15,000 subjects who aged 50 years and above (Chew *et al*., 2018).

1.2 Diabetic Macular Oedema and Its Treatment

Diabetic macular oedema(DMO) is an advanced manifestation of DR that has become one of the leading causes of vision damage in the population of working age of diabetic patients (Miller and Fortun, 2018). It was reported that there were about 7.6% of patients currently had been diagnosed with DMO among diabetic patients worldwide (International Diabetes Federation, 2017a). The highest prevalence of DMO was 11% in the Eastern Mediterranean region, then 8.9% in the European region and the lowest prevalence was 5.6% in the Western Pacific region where Malaysia is one of the country included in this region (International Diabetes Federation, 2017a). Previously, the prevalence of diabetic patients who had been diagnosed with DMO was 6.8% (Yau *et al.*, 2012). As DMO can occur in any stage of DR, it may contribute to the risk of blindness in diabetic patients. Blindness causes functional limitation to the patients to perform self-care activities that can help them to control their blood glucose level such as exercising, preparing healthy meals, taking insulin and medications (Siersma *et al.*, 2019). Also, these patients may experience mental

distress, loneliness and social limitation such as unemployment and the ability to utilise healthcare services (Fenwick *et al.*, 2012). It was reported globally that 64% of DMO patients experience limitations on performing their daily activities due to visual deterioration which that give some subsequent impact on their quality of life (International Diabetes Federation, 2017a).

Diabetic macular oedema is characterised by a thickening of the macular region of the retina due to an accumulation of fluid and protein from leaking blood vessels (Zhang *et al.*, 2014). The pathogenesis of DMO is not clearly explained but it appears that, microvascular obstruction may lead to retinal ischemia that causes the upregulation of intraocular levels of vascular endothelial growth factor (VEGF). As a result, this may lead to many critical physiologic processes such as vascularization, increased vascular permeability, and the production of proinflammatory cytokines in diabetic patients *(*Boyer *et al.,* 2013*).* The VEGF is considered as a crucial cytokine in the development and progression of DMO *(*Boyer *et al.,* 2013*).* Therefore, its clinical blockade or neutralisation of the VEGF by intraocular injections of anti-VEGF may help to reduce the vascular leak in the macula.

Beside metabolic control, there are other treatment options for patients with DMO which include laser photocoagulation, intravitreal injections of steroid and intravitreal anti-VEGF (Boyer *et al.*, 2013). Laser photocoagulation is currently the treatment gold standard to prevent vision loss among DMO patients (Cheung *et al.*, 2018). While, intravitreal injections such as steroid and anti-VEGF have been used to improve the vision of the patients (Cheung *et al.*, 2018). Among these two, treatment with steroid is less preferred due to its side effect of glaucoma and cataract.

For patients with refractory DMO or patients who are not responding to any of the above treatment options, they need to undergo vitreo-retinal surgery. Anti-VEGF agents are currently considered as the latest treatment options, and there are two anti-VEGF available to be used for the treatment of DMO (Cheung *et al.,* 2018). These agents are Ranibizumab and Aflibercept.

This study is focusing on the intravitreal Ranibizumab (IVR), a recombinant humanised monoclonal antibody fragment which binds to all isoforms of VEGF specifically VEFG-A. It inhibits VEGF-A from binding to its receptors (Rs) VEGFR-1 and VEGFR-2 (Boyer *et al.,* 2013). This antiangiogenic therapy decreases the vascular permeability in DMO, causing changes of central macular thickness (CMT) and thereby improvement in visual acuity (VA). As a result, this positive effect of IVR may help to improve vision and prevent further visual loss (Pieramici *et al.,* 2016). Intravitreal Ranibizumab (IVR) has been approved by the European Medicines Agency (EMA) in 2011 and US Food and Drug Administration (FDA) in 2012 for DMO treatment because of its significant efficacy over other treatment alone. In Malaysia, IVR has just recently being listed in the Ministry of Health Drug Formulary for the treatment of DMO in the public sector in 2015.

1.3 Problem Statement

The prevalence of DMO is expected to increase rapidly worldwide in line with the increasing prevalence of DM due to ageing of the population, urbanisation, increasing of life expectancy of those with diabetes and also increasing the prevalence of obesity and physical inactivity (Jan Mohamed *et al.*, 2015). As Malaysia is expected to follow the same trend of other countries, continuous improvement in treatment for DMO has become increasingly important as well (Jan Mohamed *et al.*, 2015). In Malaysia, treatment of DMO with IVR is considered new as it was approved to be used only by a retinal specialist. Initially, patients will be received three monthly injections of IVR 0.5 mg and then monitor for improvement in terms of VA and the presence of macular oedema. The additional injection may then be given monthly if the patient does not respond well to the treatment and this means they must be regularly reviewed in the clinic. For some patients, repeated injections of IVR were not always possible because it can be burdensome in term of inconvenience, follow-up and cost to the patients and health care provider (Chen *et al.*, 2010). Furthermore, these patients may still have the macular oedema in the eyes even after receiving further intravitreal injections of anti-VEGF. Moreover, there were inconsistent and inconclusive findings in the previous studies about the associated factors that may influence the treatment effect in term of CMT.

1.4 Justifications and Benefit of the study

The DMO is one of the main reasons that contribute to the estimation of 1 out of every 39 people had blindness, and 1 out of every 52 people had visual impairment due to DR in 2010 (Leasher *et al.*, 2016). As anti-VEGF become the most common treatment after laser treatment, continuous evaluation of its treatment effect on improving

the vision of diabetic patient may assist in enhancing the quality of life and prevent severe consequences of the complications. In western countries, several controlled clinical studies have demonstrated that IVR therapy results in resolution of macular oedema and improved VA outcomes compared with focal or grid laser alone. However, the outcome measures were estimated at a range of follow up from 6 to 24 months, and patients received more than three anti-VEGF injections (Nguyen *et al.*, 2009; Massin *et al.*, 2010; Mitchell *et al.*, 2011; Nguyen *et al.*, 2012; Ishibashi *et al.*, 2015)

Some patients may respond poorly to Ranibizumab in daily clinical practice despite the positive evidence reported in the literature. Many other factors possibly influence the patient's potential response to treatment. The previous studies showed inconsistent findings of the associated factors that influence the treatment effect. The difference in population profile, geographical area and the management of patient's care might be related to this inconsistency findings. Further understanding and identifying the treatment response and its associated factors of anti-VEGF therapy may help the ophthalmologists in decision making in the treatment of DMO, reduce their burden in term of inconvenience, follow-up and cost to the patients and health care provider and consequently help to improve the quality of life and prevent severe consequences of DMO. Therefore, due to inadequacy of study that evaluates the short-term effects after first three IVR and the associated factors that can influence the treatment response in DMO patients prompted us to carry out this study.

1.5 Research Questions

1. What is the mean change of CMT after three injections of IVR among DMO patients?
2. What is the proportion of optimal treatment response after three injections of IVR among DMO patients?
3. What are the factors that influence the changes in the CMT among DMO patients on IVR?

1.6 Research Objectives

1.6.1 General Objectives

To evaluate the changes of CMT and factors associated with the changes among DMO patients on IVR

1.6.2 Specific Objectives

1. To estimate the mean changes of CMT after three injections of IVR among DMO patients
2. To estimate the proportion of optimal treatment response after three injections of IVR among DMO patients
3. To identify associated factors of changes of the CMT after three injections of IVR among DMO patients.

1.7 Research Hypotheses

Socio-demographics, treatment factors and clinical characteristics are associated factors that influence the changes of CMT in DMO patients on IVR.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This section explained an overview of literature related to DMO and treatment options in DMO focusing, on the anti-VEGF, IVR. In addition, discussion includes a review of factors that may influence the treatment outcomes of IVR in DMO patients. This literature review will describe all the results that have been reported and demonstrated in previous studies related to the treatment of DMO patients.

2.2 Search Terms and Databases

This literature search of electronic databases was undertaken to include various databases such as Scopus, Science Direct, EBSCOhost, PubMed and Google Scholar for relevant articles regardless to the type of articles. The search included DMO, Ranibizumab, Lucentis, CMT, prevalence, epidemiology, blindness and Malaysia were used in combination using Boolean operators. Selection of information in the articles was based on its relevance to the present study.

Table 2.1: Results of search term in respective database

Search Term	Database				
	Scopus	**Science Direct**	**EBSCO host**	**Pub Med**	**Google Scholar**
("Diabetic Macular Edema" OR "Diabetic Macular Oedema")	6264	3951	6660	4039	17700
("Diabetic Macular Edema" OR "Diabetic Macular Oedema") AND ("Malaysia")	1	34	68	9	704
"(Diabetic Macular Edema" OR "Diabetic Macular Oedema") AND (“Prevalence") AND ("Blindness")	95	675	871	44	805
("Diabetic Macular Edema" OR "Diabetic Macular Oedema") AND ("Ranibizumab" OR "Lucentis")	1438	1161	1963	712	13300
("Diabetic Macular Edema" OR "Diabetic Macular Oedema") AND ("Ranibizumab" OR "Lucentis") AND ("Central Macular Thickness”) AND (“Factors”)	78	131	218	10	1550

2.3 Central Macular Thickness

The human eye consists of several main components including the cornea, pupil, iris, lens, retina and optic nerve, which are responsible for providing vision. Each component has an important role in maintaining normal visual function. When the light enters the eye, it is focussed through the cornea and passes it into the iris. The size of the pupil will be adjusted by the iris so that the amount of light reaching the back of the eye can be controlled. Then, the lens helps to refract incoming light and focus it onto the retina. The visual information will then be transmitted to the brain through the optic nerve before getting the vision (Kierstan and David, 2018). An oval-shaped pigmented area known as the macula, located in the centre of the retina is responsible for giving sharp, clear and central vision (Figure 2.1).

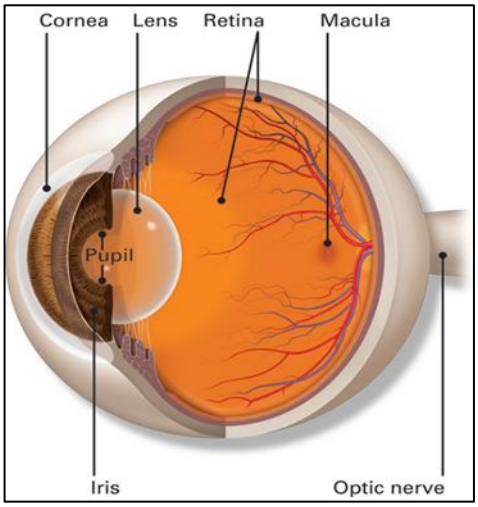

Figure 2.1: Basic parts of the human eye
Source: (Kierstan and David, 2018)

CMT is defined as the thickening within 1 mm from the centre of fovea that can be measured by Optical Coherence Tomography (OCT) (Figure 2.2). The evaluation of CMT by OCT provides an objective assessment of the degree of macular oedema for early diagnosis and allow the ophthalmologist to monitor the treatment efficacy for DMO (Hannouche *et al.*, 2012). A study reported that in healthy eyes, the normal thickness of the central macular was measured to be 270.2 µm using spectral-domain OCT (Grover *et al.*, 2009). A previous study found that the macular thickness in diabetic patients is usually greater than healthy individuals and the variations are due to different degrees of oedema (Hannouche *et al.*, 2012). The changes in CMT after treatment of DMO were considered to be the primary outcome measure reported in many previous studies related to DMO (Bong *et al.*, 2016).

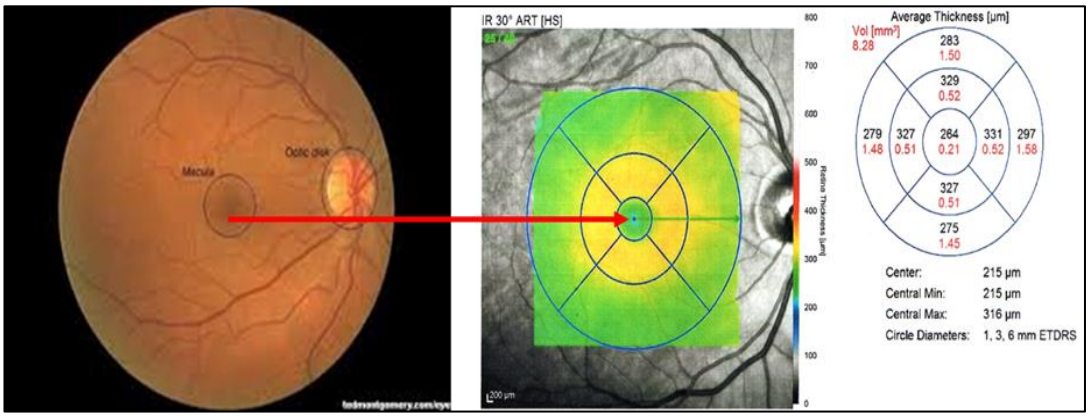

Figure 2.2: Macular thickness map by OCT

Source:(Maine Eye Center, 2016)

2.4 Diabetic Macular Oedema

DMO is one of the diabetic eye diseases manifested due to microvascular complication of DM, which commonly lead to vision loss among diabetic patients with DR (Mitchell *et al.*, 2012). This complication is thought to be caused by metabolic abnormalities, including chronic hyperglycaemia, diabetic dyslipidaemia, hypertension and vascular inflammation (Miller and Fortun, 2018).

2.4.1 Clinical Feature of Diabetic Macular Oedema

This condition is characterised by retinal swelling due to accumulation of fluid and protein in the macula of the eye, and associated with deposition of lipid in retinal layer which is derived from leaking retinal vessels known as hard exudates. The clinical manifestations of DMO include poor vision and distortion of the image, which can be further classified as mild, moderate and severe DMO. The severity of DMO was considered as mild when the oedema was in posterior pole but a distance from the centre of the macula, moderate DMO when oedema approaching the centre of the macula and severe DMO when oedema was present in the centre of the macula (Figure 2.3) (Wu *et al.*, 2013). This disease is usually asymptomatic at its earliest stage but as the disease progress, and if without any treatment, patients may experience progressive vision loss over time (Lee *et al.*, 2015). Therefore, diabetic patients need to undergo a regular eye examination to identify and detect any ocular abnormalities that can be treated early before significant vision loss occurs.

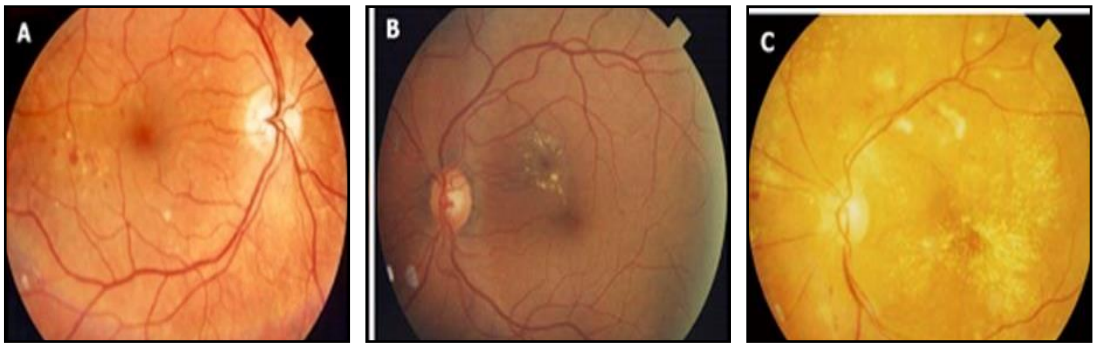

Figure 2.3: Diabetic macular oedema A: Mild; B: Moderate; C: Severe
Source: (Wu *et al.*, 2013)

2.4.2 Pathophysiology of Diabetic Macular Oedema

A thin layer of light-sensitive tissue which is lining at the back of the eye is known as the retina. The retina is divided into two main parts, retinal pigment epithelium (RPE) and neurosensory retinal layers (Romero-Aroca *et al.*, 2016). RPE is the outermost monolayer cells contains melanin pigment in the cytoplasm that allows absorption of light to reach the photoreceptor of the retina. While, neurosensory retinal consists of several layers including photoreceptor which consists of the ellipsoid zone and external limiting membrane (ELM), outer nuclear, outer plexiform, inner nuclear, inner plexiform, ganglion cell and nerve fibre layer that allows transmission of an impulse from photoreceptors to the thalamus of the brain (Figure 2.4).

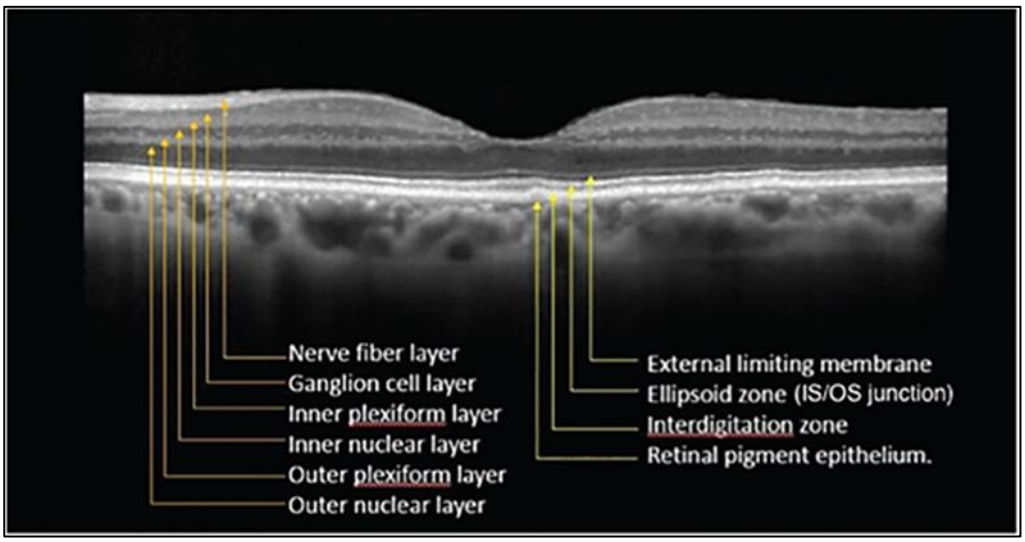

Figure 2.4: Cross-sectional retinal layer imaged by OCT

Source : (Mohandass, 2017)

DMO is mainly caused by the disruption of the blood-retinal barrier due to several causes such as sustained hyperglycaemia in diabetes patient leading to retinal ischemia followed by several mechanisms involved including increased in vascular permeability, cytokine activation, altered blood flow, hypoxia and inflammation (Akiyode and Dunkelly-Allen, 2015; Romero-Aroca *et al.*, 2016). Studies have reported that the major contributory factor to angiogenesis and permeability in this inflammatory process is the VEGF (Agarwal *et al.*, 2014). The activation of VEGF can be stimulated by the hypoxia condition caused by the microvascular injury (Romero-Aroca *et al.*, 2016). The VEGF is considered a key cytokine in the development and progression of DMO which promote vascular permeability via the activation of its receptor found on the surface of endothelial cells.

The elevation of VEGF level increases the vascular permeability by loosening the tight junction between endothelial cells and the wall of capillaries. Subsequently, as the junction loosen, the vascular leakage of fluid and serum proteins, plasma constituents and lipids into the surrounding tissue of retina particularly the centre of the macula, the portion of the retina responsible for sharp and central vision. Leakage fluid from vascular permeability allowing the accumulation of the intracellular and extracellular fluid that leads to the formation of macular oedema (Romero-Aroca *et al.*, 2016). This condition may cause an increase in CMT that can be measured by OCT (Romero-Aroca *et al.*, 2016) (Figure 2.5).

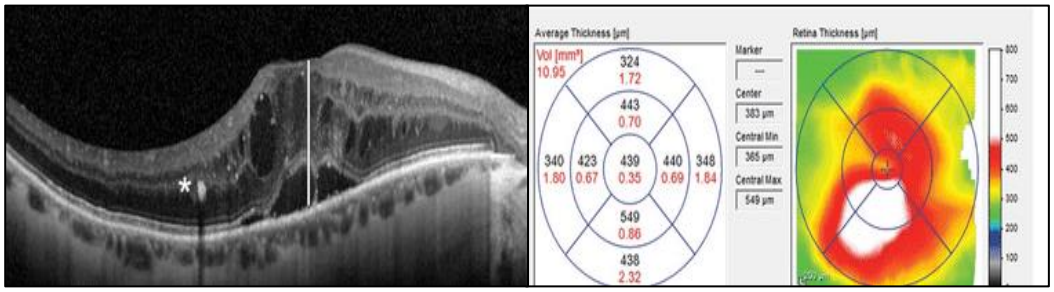

Figure 2.5: Evaluation of macular thickness by OCT

Source: (Muftuoglu *et al.*, 2017)

Excessive VEGF may also stimulate the growth of new, abnormal retinal blood vessels and capillaries to provide more oxygen to the eye due to hypoxia. However, these new blood vessels are fragile and prone to torn and bleed, causing leaking of fluid and proteins as well (Miller and Fortun, 2018). Over time, this abnormal increase of fluid volume and the imbalance between fluid entry and exit promote the formation of macular oedema and the deposit of hard exudate, both of which disrupt the morphology of retina. This disruption may lead to permanent retinal structural damages causing visual loss and blindness in DMO patients (Amoaku *et al.*, 2015; Miller and Fortun, 2018).

2.4.3 Risk Factors of Diabetic Macular Oedema

The prevalence of DMO may increase with increasing duration of diabetes. A study showed that patients with more than ten years duration of DM have 8.51 higher odd to have DMO than patients with less than ten years duration of DM (Varma *et al.*, 2014). The Wisconsin study reported that diabetic patients with disease duration of 20 years or more frequently experience macular oedema as the main cause for visual loss (Yau *et al.*, 2012). The increase duration of diabetes indicates that the incidence of retinopathy may progress from mild non-proliferative diabetic retinopathy (NPDR) to proliferative diabetic retinopathy (PDR) and subsequently lead to the development of

DMO at any stages of retinopathy (Lee *et al.*, 2015). Another study showed that DMO patients who have a longer duration of diabetes have higher mean values for ganglion cell complex (GCC) parameters which were strongly associated with the CMT. GCC is a parameter used to measure whether the retinal structures of the eye were affected with long-standing DMO. Higher values of GCC parameters means more structural damage has occurred to the macula, which emphasises the need for early treatment before irreversible damage happens (Refai and Hassan, 2018). In term of glycosylated haemoglobin (HbA1c), several studies had reported that elevated HbA1c might increase the risk of diabetic patients to develop DMO (Varma *et al.*, 2014; Liu *et al.*, 2017).

2.4.4 Prevention of Diabetic Macular Oedema

The risk of DMO and progression of retinopathy can be reduced by intensive metabolic control (Peng and Tsai, 2018). Apart from metabolic control of hyperglycaemia, hypertension and hyperlipidaemia, early eye screening is critically important for early detection of DMO in order to prevent severe visual disability and to identify those at risk of losing vision among diabetic patients (Agarwal *et al.*, 2014). At the time of patients were diagnosed with type 2 diabetes, it was recommended that eye examination was performed and repeated annually. Then, a less frequent eye examination can be performed after one or more normal eye examination, especially in patients with well-controlled DM (American Diabetes Association, 2019).

2.4.5 Treatment Options of Diabetic Macular Oedema

Prevention of severe visual disability or blindness is the major goal in the treatment of DMO. There are several treatment options available for DMO including laser, corticosteroid, anti-VEGF and surgical treatment. Laser treatment has an important role in preserving vision, especially in patients with macular oedema, which does not involve the fovea (Yorston, 2014). In addition, the laser treatment should be given first before anti-VEGF is administered if new vessels are observed in DMO patient (Yorston, 2014). The laser treatment is considered to be much convenient for the patients as it is effective as a single treatment as compared to anti-VEGF, which require repeated monthly injection (Yorston, 2014). Laser treatment, however, has a lack of ability to improve visual acuity (VA) and may cause peripheral and night vision loss (Sayin *et al.*, 2015). The corticosteroids which have been reportedly effective to be used in DMO is triamcinolone acetonide (Fung *et al.*, 2020) . It is administered by intravitreal injection or posterior subtenon route in DMO. The important role of steroids in DMO is to improve vision. However, the limitations of the steroids in DMO include causing the formation of cataracts and increased intraocular pressure which may lead to infection (Sayin *et al.*, 2015). In this study, the main focus is mainly on the anti-VEGF, specifically IVR.

2.5 Anti-Vascular Endothelial Growth Factor Drugs

The anti-VEGF drugs have been recommended as the first-line treatment for diabetic patients with centre involving DMO with vision loss (Cheung *et al.*, 2018). The mechanism of action of anti-VEGF is best described as inhibition of VEGF from binding to its receptor in the eye, and thereby reduce neovascularisation and fluid build-up which lead to an improvement in vision and prevent further visual loss in DMO (Boyer *et al.*, 2013). The anti-VEGF drug is administered by an intravitreal route to gives the maximum effect only in the eye but not others. There are two regimes for administering anti-VEGF drugs, including continuous and intermittent or as required regimes. In many clinical trials, anti-VEGF is administered as a continuous regime over a specific duration of time. Although the treatment was found to be effective, it is also highly cost and inconvenient for patients and health care provider (Yorston, 2014). For the as required regime, initially, patients will be received three injections of anti-VEGF drugs given over three months and then followed by additional injection if required. In this case, some patient may or may not require additional treatment depending on the VA and the macular thickness. Past studies have shown that early intensive therapy with anti-VEGF, especially within 6 to 12 months is important to achieve a better outcome in treatment effect (Cheung *et al.*, 2018).

In term of safety, a previous systematic review reported that the use of anti-VEGF drugs had been associated with significant visual improvement with minimal serious side effects in several clinical studies (Agarwal *et al.*, 2014; Stefanini *et al.*, 2014). Similarly, a previous three-year study period reported that anti-VEGF was generally well tolerated with no safety concerns apart from frequently reported cataracts and nasopharyngitis in 16.3% and 23.3% of patients respectively

(Schmidt-Erfurth *et al.*, 2014). A meta-analysis on systemic safety of prolonged monthly anti-VEGF for DMO, on the other hand, shows that high-risk DMO patients who received two years of monthly anti-VEGF treatment may have a possible increased risk of cerebrovascular events and even death (Avery and Gordon, 2016). As such, consideration of total exposure to anti-VEGF agents is crucial, particularly when treating high-risk patients for vascular disease.

2.6 Efficacy of Intravitreal Ranibizumab

The IVR is the first intravitreal anti-VEGF used in the management of DMO. The efficacy of IVR in the reduction of macular thickness has been demonstrated in multiple randomised, controlled clinical trials, including the READ 2, DRCR.net Protocol I, RESOLVE, RESTORE, REVEAL, RIDE and RISE study. A READ-2 multicentre, three-arm prospective randomised clinical trial conducted in the USA was the first large RCT (n=126) which demonstrated a significant reduction in CMT in all three groups at six months follow-up. However, the reduction was higher in IVR alone group which was reduced by 50%, as compared to laser alone and a combination of IVR and laser group which reduced by 33% and 45% respectively. Furthermore, this study showed that the addition of laser did not give any advantages in further reduction of the macular thickness (Nguyen *et al.*, 2009).

In 2010, a multicentre four-arm placebo-controlled RCT known as Diabetic Retinopathy Clinical Research Network study had compared IVR 0.5 mg plus prompt (within 3–10 days post-IVR) or deferred (≥24 weeks) laser with sham injection plus prompt laser, or triamcinolone 4 mg plus prompt laser (n=854). The study reported that IVR or triamcinolone combined with either prompt or deferred laser

photocoagulation group have a similar reduction in macular thickness but greater than sham plus prompt laser group alone at 1-year follow-up point (Elman *et al.*, 2010). Therefore, this study showed that a combination of IVR with laser is more effective than laser alone to treat DMO, which involved the central macula for at least one year.

Another multicentre placebo-controlled RCT by RESOLVE study group (n=151) demonstrated that those in the IVR group after three monthly injections and subsequently as needed has a higher reduction of CMT as compared to the sham group at months 12. The mean (SD) reduction of CMT for IVR and sham group were 194.2 (135.1) µm and 48.4 (153.4) µm respectively (Massin *et al.*, 2010). Similarly, in the multicentre phase III RESTORE study (n=345), IVR 0.5 mg which was given three monthly injections and subsequently as needed, either alone or combined with laser therapy showed a significantly greater mean reduction in CMT from baseline as compared with laser alone. The mean reduction of CMT for IVR alone, IVR plus laser and laser alone were 118.7 µm, 128.3 µm and 61.3µm respectively at 12 months follow-up. However, this study showed there was no difference in efficacy perceived between the two IVR groups (Mitchell *et al.*, 2011).

The RISE (n=377) and RIDE (n=382) studies were two parallel phases III, multicentre, double-masked, sham injection-controlled, randomised studies conducted in the United States and South America. The study arms are similar to those in the RESOLVE study, where patients received monthly injections of 0.3 or 0.5 mg IVR or sham injections. These studies reported that reduction of CMT was statistically higher in IVR treated group as compared to the sham group. Furthermore, the proportion of patients with CMT $\leq$ 250 µm in IVR 0.5 mg (76%) and IVR 0.3 mg (74.4%) was

significantly higher as compared to the sham group (43.3%) (Nguyen *et al.*, 2012). After 24 months, further 12 months follow up was conducted in the RISE and RIDE trials. Patients who previously received sham injections were eligible to receive monthly IVR 0.5 mg, and patients originally randomised to monthly IVR administration of 0.3 or 0.5 mg continued to receive their assigned dose. At month 36, the average mean CMT thickness for IVR 0.3 mg, IVR 0.5 mg and the sham group was 223.4 µm, 201.9 µm and 194.1 µm respectively (Brown *et al.*, 2013).

In the Asian population, a phase III multicentre double-masked clinical trial was conducted on patients with visual impairment resulting from DMO. At months 12, this REVEAL study (n=396) suggested that IVR monotherapy or combined with laser showed a significant reduction in retinal thickness over laser treatment alone. Furthermore, the addition of laser treatment to IVR was not found to give any further benefit (Ishibashi *et al.*, 2015). Another prospective study (n=63) which compared the efficacy of IVR with bevacizumab reported that there was a significant reduction in mean CMT in both groups as compared to baseline. However, no significant difference in term of reduction of CMT even though the number of injections was slightly higher in bevacizumab than in the IVR group (Nepomuceno *et al.*, 2013).

2.7 Factors Associated with Changes of Central Macular Thickness

Although several studies have been conducted to find the associated factors that may influence the effectiveness of IVR in DMO in term of CMT changes, their findings were inconsistent with each other. These associated factors can be classified as either systemic or ocular factors and are elaborated in the section below.

2.7.1 Systemic Factors

2.7.1.1 Age

A previous study found that DMO patients with younger age tended to have a greater change in CMT (p=0.022) after three consecutive monthly injections of IVR (Lai *et al.*, 2017). However, there was no significant difference in CMT changes after adjustments for confounding factors (Lai *et al.*, 2017). The long-term outcomes of the RISE and RIDE studies between younger-aged and older patients showed the changes of CMT in these group of patients was less likely to achieve less than 250 µm (Sophie *et al.*, 2015).

Similarly, another study conducted by the Diabetic Retinopathy Clinical Research Network identified that there was no significant difference in term of changes of CMT (p=0.260) between those who are younger than 60 years old and those who are 60 years old and above at month 12 (Bressler *et al.*, 2012). Also, a study done by Sato *et al* (2017) reported that the effect of IVR treatment was not associated with patient age in term changes of CMT. This relationship between age and changes of CMT may be related to the reduction in retinal cell functioning, thickening of internal limiting membrane and changes in RPE (Salvi *et al.*, 2006).

2.7.1.2 Sex

It was reported in the literature that men with DMO had thicker CMT than women with DMO (Arthur *et al.*, 2019). However, a previous retrospective study reported that sex was not related to changes of CMT at month 3 of IVR treatment (Lai *et al.*, 2017). Similarly, a study showed that sex was also not significantly related to the changes of CMT after 24 months of IVR treatment (Sophie *et al.*, 2015).

2.7.1.3 Ethnicity

It was reported that CMT varied significantly with ethnicity (Grover *et al.*, 2009). These findings were consistent with the previous study which demonstrated that participants from African American have significantly greater changes of CMT by 27.3 µm as compared to white participants after treated with anti-VEGF at month 24 (Bressler *et al.*, 2019). But to the best of our knowledge, the association between ethnicity and changes of CMT among Asian population after treated with anti-VEGF have not been previously reported.

2.7.1.4 Duration of Diabetes Mellitus

A previous study reported that DMO patients with a shorter duration of diabetes who received IVR injection, the changes of CMT in these group of patients were less likely to achieve less than 250 µm (Sophie *et al.*, 2015). Although, a study done by Bressler *et al* (2012) reported that the median changes of CMT in patients with duration of DM < 15 years was slightly higher (151 µm) as compared to patients with duration of DM ≥ 15 years (103 µm), there was no significant difference in changes of CMT between these two groups. The study findings may also indicate that the duration of diabetes may correlate well with the severity of DR (Sophie *et al.*, 2015). As the duration of DM increase, it may worsen the DR progression especially in poorly controlled diabetic patients.