COMPARISON OF MACULAR THICKNESS, RETINAL NERVE FIBER LAYER THICKNESS AND OPTIC NERVE HEAD PARAMETERS IN HBE/ BETA THALASSEMIA AND CONTROL

BY

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

(OPHTHALMOLOGY)

SCHOOL OF MEDICAL SCIENCES

UNIVERSITI SAINS MALAYSIA

2016

Disclaimer

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

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ACKNOWLEDGEMENTS

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

I wish to express my sincere appreciation and gratitude to my supervisors, Associate Professor Dr. Adil Hussein, Consultant Ophthalmologist and senior lecturer at HUSM, Dr. Suresh Kumar A/L Vasudevan, Consultant Ophthalmologist at Hospital Sultanah Aminah, Johor Bharu and my co-supervisor Dr. Khairy Shamel Bin Sonny Teo, senior lecturer at HUSM for their undivided guidance, attention to details and direction throughout this entire dissertation. Without their supervision, insightful comments and encouragement, this dissertation would not have been fruitful.

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

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

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LIST OF ABBREVIATIONS

ACDR	Average Cup Disc Ratio
AMD	Age Macular Degeneration
α	Alpha
β	Beta
BMO	Bruch's Membrane Opening
CHF	Congestive Heart Failure
CNS	Central Nervous System
CRVO	Central Retinal Vein Occlusion
CSLO	Confocal Scanning Laser Ophthalmoscopy
HbA	Hemoglobin Adult
HbF	Fetal Hemoglobin
HbE	Hemoglobin E
HbE/ β-thalassemia	Hemoglobin E/ β-thalassemia
ICA	Iron Chelating Agent
IDA	Iron Deficiency Anaemia
ILM	Internal Limiting Membrane
IOP	Intraocular Pressure
mRNA	Messenger Ribonucleic Acid
NAION	Non-Arteritic Anterior Ischemic Optic Neuropathy
NTDT	Non Transfusion Dependent Thalassemia

NTG	Normal Tension Glaucoma
OCT	Optical Coherence Tomography
ON	Optic Nerve
ONH	Optic Nerve Head
OSAS	Obstructive Sleep Apnea Syndrome
PCA	Posterior Ciliary Artery
PS	Phosphatidylserine
RBC	Red Blood Cell
RNFL	Retinal Nerve Fiber Layer
RPE	Retinal Pigment Epithelium
SD-OCT	Spectral Domain Optical Coherence Tomography
SEA	South-East Asia
SLP	Scanning Laser Polarimetry
SPCA	Short Posterior Ciliary Artery
SS-OCT	Swept Source Optical Coherence Tomography
TD-OCT	Time Domain Optical Coherence Tomography
TEE	Thromboembolic Event
VCDR	Vertical Cup Disc Ratio

ABSTRAK

Pengenalan

HbE/ talasemia beta merupakan salah satu daripada hemoglobinopati yang biasa ditemui di Asia Tenggara dan kini telah menjadi satu masalah kesihatan awam yang kritikal. Selain daripada anemia patologikal, ia juga mempunyai ciri-ciri utama lain seperti hepatosplenomegali dan deformasi struktur rangka tulang kraniofasial. Biometri okular juga turut terjejas akibat kelainan pada tumbesaran rangka tulang orbit. Selain itu juga, terdapat ciri-ciri lain seperti perubahan pada struktur saraf retina dan optik yang berlaku pada pesakit ini.

Objektif

Membandingkan purata ketebalan makula, ketebalan serat saraf retina dan parameter kepala saraf optik antara kumpulan pesakit HbE/ talasemia beta dengan kumpulan kawalan.

Metodologi

Satu kajian rentas telah dijalankan di Hospital Universiti Sains Malaysia bermula dari bulan Jun tahun 2014 sehingga bulan Mac 2016. Pesakit yang telah disahkan dengan HbE/ talasemia beta dan populasi yang normal dan sihat telah dipilih berdasarkan ciri-ciri inklusi dan eksklusi kajian. Data ketebalan makula, ketebalan serat saraf retina dan parameter kepala saraf optik telah diukur

menggunakan Cirrus HD-OCT. Analisa statistik telah dilakukan dengan menggunakan perisian SPSS versi 22.0

Keputusan

Sejumlah 132 peserta telah terlibat dalam kajian ini (66 pesakit HbE/ talasemia beta dan 66 subjek kawalan). Tiada perbezaan yang signifikan dalam purata ketebalan makula bagi kedua-dua kumpulan. Tiada perbezaan signifikan pada purata ketebalan serat saraf retina. bagi kumpulan HbE/ talasemia dan kumpulan kawalan. Tiada perbezaan yang signifikan turut didapati untuk parameter kepala saraf optic bagi kedua-dua kumpulan.

Kesimpulan

Kajian ini telah menunjukkan tiada perbezaan signifikan pada purata ketebalan macula, purata ketebalan serat saraf retina dan parameter kepala saraf optic bagi kumpulan HbE/ talasemia dan populasi yang normal.

ABSTRACT

Introduction

HbE/ β-thalassemia is one of the hemoglobinopathy that commonly found in South East Asia (SEA) and it has become a severe public health problem today. Apart from the pathological anemia, they also share other common features like hepatosplenomegaly and craniofacial bony deformities. Ocular biometry can be affected as well due to abnormal growth of the orbital bone. Other than these, structure of the retina and the optic nerve may be altered in these patients.

Objective

To compare mean macular thickness, retinal nerve fiber layer (RNFL) thickness and optic nerve head (ONH) parameters between HbE/ β-thalassemia group and control.

Methods

This is a cross-sectional study conducted in Hospital Universiti Sains Malaysia from June 2014 till March 2016. Patients with confirmed HbE/ β-thalassemia and healthy population were selected based on the inclusion and exclusion criteria. Baseline macular thickness, RNFL thickness and ONH parameters were measured by using Cirrus HD-OCT. Statistical analysis was performed using SPSS version 22.0.

Results

A total of 132 participants were recruited (66 participants in HbE/ β-thalassemia group and 66 participants in control). There was no significant difference in mean macular thickness in between the two groups. No significant different found in the mean RNFL thickness in between HbE/ β-thalassemia and control. There was also no significant difference in ONH parameters between two groups.

Conclusion

The study revealed that there was no significant differences in both mean macular thickness, RNFL thickness and ONH parameters in between HbE/ β-thalassemia and normal population.

Chapter 1

Introduction

1.1 THALASSEMIA

Thalassemia was derived from 2 Greek words; Thalassa (sea) and Anemia. In the early 20th century, most of the cases were reported in children of Mediterranean origin, therefore Thalassa alluded to the Mediterranean Sea (Whipple and Bradford, 1936). Thalassemia, in other words, is also known as Mediterranean anemia. It is an autosomal recessive inherited hemoglobin disorder. According to evidences in research, HbA and HbF consist of 2 α chains which are combined with a β, delta or gamma chains (Cao and Galanello, 2010). As reported by Weatherall (1998), hemoglobinopathies are disorders which can be traced to mutations within specific globin genes including α or β which can cause ineffective erythropoiesis. Based on the types of globin defects, the thalassemia can be divided into α and β. Alpha-thalassemia occurs when a gene or genes related to the α-globin proteins are missing or mutated. Whereas, β-thalassemia occurs if a gene or genes related to the β-globin proteins are involved and result in deficiency of β-globin chain synthesis (Thein, 2005).

1.2 HEMOGLOBIN E

HbE is an abnormal β-hemoglobin variant. This HbE allele structural variant has a substituted amino acid at position 26, from glutamic acid to lysine (Olivieri et al, 2011), which results in a structurally abnormal hemoglobin molecule, further resulting in abnormal processing of mRNA (Orkin et al, 1982). This mRNA is non-functional as a result of an associated generation of a new-stop codon (Datta et al, 2006). Evidences in research further concluded that such abnormal processing can result in multiple pathophysiological conditions which are aimed at reducing the β chain synthesis. This can result in limited erythropoiesis as well as

associated with the shortening of red cell survival (Pootrakul et al, 2000). Hence, individuals with HbE are characterized with a mild form of β-thalassemia (Olivieri et al, 2011).

1.2.1 Epidemiology of Hemoglobin E

HbE populations are commonly found in SEA especially in Cambodia, Thailand and Laos, where the prevalence can range from 25% to 60%; the distribution of HbE in Malaysia ranges from 4% to 45% of the population (Weatherall and Clegg, 2001; Fucharoen and Winichagoon, 1997). Due to the population migration, they can also be found in the countries other than SEA, such as Bangladesh, Sri Lanka and Nepal (Weatherall and Clegg, 2001; Fucharoen and Winichagoon, 1997). The carrier rate of HbE in SEA is high especially in Cambodia, Laos, and Thailand, which ranges from 4% to 80%; in Malaysia, it has been estimated as 3% to 40% (Weatherall and Clegg, 2001).

1.3 HEMOGLOBIN E/ BETA THALASSEMIA

This disorder is found to occur as a result of the co-inheritance of the β-thalassemia allele from one parent and the HbE structural variant from the other parent (Agarwal et al, 1997). Extant literatures have reported that HbE/ β-thalassemia is represented by 50% of all individuals who have been affected by severe thalassemia (Fucharoen and Winichagoon, 1997; Modell and Darlison, 2008). The presence of this disorder is more common in SEA including Cambodia and Thailand where individuals inherit the allele for HbE and β-thalassemia (Weatherall and Clegg, 2001). In Malaysia, George (2013) added the Malays and Orang Asli populations are the 2 most common groups in peninsular Malaysia that suffer from HbE/ β-thalassemia. Based on a cohort study by Hanafi et al, (2014), HbE/ β-

thalassemia can be classified phenotypically into HbE/b^0 and HbE/b^+, where b^0 is without a β-globin chain synthesis; b^+ with some degree of β-globin chain synthesis. Clinically, the severity of the HbE/ β-thalassemia can be grouped into mild, moderate and severe based on a new scoring system (Sripichai et al, 2008). The scores (0-10) are obtained based on few parameters including age of presenting illness, age of receiving the first transfusion, frequency of transfusion, level of the hemoglobin, spleen size, and the status of growth and development in HbE/ β-thalassemia patients (Sripichai et al, 2008). With the assistance of this new classification system, Sripichai et al, (2008) found that the HbE/ β-thalassemia patients were almost equally distributed into 3 groups with different severity.

1.3.1 Clinical Features of HbE/ β-Thalassemia

According to Weatherall (2000), the clinical features of HbE/ β-thalassemia can range from minor to major. As Fucharoen and Winichagoon (2000) reported those individuals who are most severely affected are transfusion dependent and show signs of intermittent jaundice, possible retardation of growth, expansion of the bone marrow cavity and more importantly hepatospenomegaly. Another common feature in severe β-thalassemia is craniofacial bony deformities which comprises of bossing of the skull, depression of the nasal bridge, prominent malar eminence, hypertrophy of the maxillae and mongoloid slant of the eye. However, Olivieri et al (2008) reported that those individuals who are less severely affected and not on regular transfusion are also found to exhibit many of these symptoms. Although the pathogenesis of bone changes is not completely understood, it is believed to relate with an abnormal turnover in bone mineral resulting from increased resorption and suppression of osteoblast activity (Morabito et al, 2004). Besides, more than 20% of the HbE/ β-thalassemia patients were reported to have respiratory tract infections; about 10% of them had

complications with CHF and some may get chronic leg ulcer, bone pain, septicemia and even transfusion related CNS complication such as cerebral haemorrhage (Fucharoen et al, 2000). The death is either caused by the disease or treatment related complications. It was reported that CHF and septicemia were the main cause of death, and about 65% of the HbE/ β-thalassemia patients die in the 3rd to 4th decade of their life (Fucharoen et al, 2000).

1.3.2 Treatment of HbE/ β-Thalassemia

Evidences in research indicate that the availability of cure for the disorder is not evident and that the only recourse for individuals with this disorder is through frequent blood transfusion, iron chelation therapy as well as splenectomy if there is presence of an enlarged spleen (Cunningham et al, 2004). Marengo-Rowe (2007) argued that chronic cases of this disorder are treated with transfusions which take place on a monthly basis. However, this can eventually increase the iron deposition in the tissue all over the body, and cause progressive organ damage (Cohen, 1987). Cunningham et al (2004) further argued that the primary cause of mortality amongst patients with this disorder is due to an iron overload as most of the transfusion programmes are accompanied by treating with an iron chelating agent. Though Bonkovsky et al (2000) reported there are alternative therapies including the use of reactivation of HbF and transplantation of bone marrow, and it is important to accept that such a reactivation occurs only in some cases.

1.4 OCULAR IMPACT

Abnormal growth of the bony orbit can cause limitation of ocular growth. Therefore, ocular biometry and refractive in severe β-thalassemia individuals can be affected. Nowroozzadeh et al (2011) found a shorter axial length, thicker crystalline lens and steeper average keratometry in their subjects. Furthermore, posterior segment of the eyes can be affected as well. The abnormalities include degeneration of the RPE, retinal vascular abnormalities, and angioid streak (Gartaganis et al, 1989). Jafari et al (2015) have reported the RPE degeneration was up to 17% in their study. The frequency of these abnormalities has been reported to increase with age (Gartaganis et al, 1989) and correlate with the number of blood transfusion (Jafari et al, 2015). According to Roy et al (2007), serious threats to vision are evident amongst patients who have thalassemia and sickle cell disorders. These patients are found to develop multi-focal areas of peripheral retinal neovascularisation. This can lead to vitreous haemorrhage.

1.4.1 Ocular Iron Deposition

Frequent blood transfusion can lead to iron deposition in all tissues in the body. The iron complexes can enter the RPE cells by binding to the surface transferrin receptors which are associated with the cytoskeleton (Hunt et al, 1989). When the excess iron complexes are transported into the cells, the hydroxyl radicals will be released through the Fenton reaction. These free radicals are harmful and toxic to the cells, as they can destroy the cell's protein, lipid, DNA, as well as disrupt the blood retinal barrier. Tawara (1986) found that the ferritin particles were mainly accumulated in the Muller and RPE cells which can cause cell degeneration. However, Jeanny et al (2013) reported that deposition of excess iron was found

in the photoreceptor segments in their animal models. Some studies showed the macular iron level in the patients with AMD was significantly higher, and they believed that this play a role in pathogenesis of AMD (Blasiak et al, 2009; He et al, 2007). Roy et al (2007) further reported that ocular complications are evident in β-thalassemia as a result of frequent blood transfusions such as retinal toxicity from the overall iron accumulation.

1.4.2 Effect of Iron Chelation Therapies

Long-term iron chelation therapies can cause an unwanted adverse effect to the eyes. Jacobs (1977) showed that there are multiple ophthalmologic changes which can occur in patients who have been diagnosed with this disorder, ranging from impact on the retina (Gartaganis et al, 1989), optic nerve (Olivieri et al, 1986; Lakhanpal et al, 1984) and decrease in the overall visual acuity as a result of Deferoxamine used. Direct RPE cells toxicity to Deferoxamine has recently been reported by Klettner et al (2010). Taher et al (2006) have further argued that patients with oral Deferiprone have higher risk of RPE degeneration than those who took Deferoxamine. However, Jafari et al (2015) reported no significant difference between the iron chelating agents in causing the RPE degeneration.

1.4.3 Optic Atrophy

Multiple case studies have shown that optic atrophy is linked to HbE/ β-thalassemia disease. Sorcinelli et al (1999) presented a case of thalassemia intermedia with reducing of the space in the optic canal, which was caused by extra-medullary hematopoietic tissue expansion. This can lead to optic nerve compression and result in optic atrophy. Furthermore, this condition was found in related to the transfusion irregularity. Ittipunkul et al (2007) have

reported another case of bilateral progressive visual loss secondary to compressive optic neuropathy in a young β-thalassemia patient; in this case, they found that the extra-medullary haematopoietic tissue was originated from the adjacent paranasal sinuses. Recently, another case presentation by Pakdel et al (2011) reported that the causes of bilateral optic atrophy not only from the compression of the intracanal portion of the optic nerve but also from the compression of the optic chiasm in thalassemia intermedia. All of these studies indicate the possible impact of the extra-medullary haematopoietic tissue growth in thalassemia intermedia patients may lead to optic atrophy.

1.4.4 Retinal Nerve Fibre Layer, Macular Thickness and Optic Nerve Head

A tissue becomes hypoxic when there is lack of oxygen supply. It can even cause tissue damage or death including RNFL when the condition is prolonged or severe. OSAS is one of the medical problems that causing prolonged and recurrent hypoxia. Study has demonstrated the RNFL thickness was significantly thinner in OSAS patients although partly can be due to the relatively increase of IOP at night during sleep, when they lie in supine position (Perez-Rico et al, 2014). Recent years Zengin et al (2014) have once again showed that the average RNFL thickness was significantly reduced in their 1 year study on OSAS patients. In β-thalassemia, due to the abnormal haemoglobin, the oxygen being transported to all the tissue including retina and ONH is reduced and result in anemic hypoxia. In such conditions, the ONH may susceptible to glaucomatous damage even at normal intraocular pressure (Marcus et al, 2001).

Furthermore, Changes in the thickness of RNFL and the macular can result from the abnormal RBCs. Study showed the RBCs in the β-thalassemia major individuals tend to

aggregate and form a large cluster of cell (Chen et al, 1996), and such changes will result in reducing of blood flow especially in the microcirculation. However, these aggregated RBCs size was found to become smaller after blood transfusion (Chen et al, 1996). Furthermore, β-thalassemia patients frequently suffer from chronic hypercoagulable state with increased incidence of thromboembolic events (Taher et al, 2008). Factors that were found to be in association with hypercoagulable state includes deficiency of protein S and protein C (Shirahata et al, 1992), increased platelet aggregation (Winichagoon et al, 1981) and increased platelet counts after splenectomy (Eldor and Rachmilewitz, 2002). Individuals with infrequent blood transfusion tend to have more platelet counts and circulating damaged RBCs, which put them in a higher risk of thromboembolism events. Taher et al (2006) reported the thromboembolic events in splenectomized β-thalassemia intermedia patients are 4 times higher than β-thalassemia major. All these abnormal changes can reduce the blood flow and perfusion pressure to the eye, which would subsequently lead to ischemic changes.

Few studies have shown significant thinning of the peripapillary RNFL thickness in some pathological anemia such as severe β-thalassemia and IDA (Aksoy et al, 2014; Turkyilmaz et al, 2013). However, there are no relevant data found in the literature that are related to the measurement of the macular thickness, RNFL and ONH parameters in HbE/ β-thalassemia.

1.5 OPTICAL COHERENCE TOMOGRAPHY

Since OCT has been introduced in the year 1991 (Huang et al, 1991), it has become one of the most important diagnostic tools in ophthalmic practice for the past decade. It is a non-invasive imaging technique that produces a cross-sectional image of the retina, RNFL and the ONH in high resolution quality. By using the concept of Michelson interferometer, OCT employs light from low-coherence source, which is directed into a fiber optic coupler and split into 2 beams; a reference beam and a sample beam. After the sample beam is backscattered from the retina, it interferes with the reference beam, and this produces an interference pattern (Fercher et al, 1988). This would enable it to detect the interface between different ocular tissues and retinal layers. According to the reflectivity changes between the different retinal layers, the RNFL thickness can be accurately measured (Huang et al, 1991; Sull et al, 2010). Other imaging techniques with different modalities such as CSLO and SLP are being used as well.

1.5.1 Time Domain Optical Coherence Tomography

The conventional TD-OCT was the first generation of OCT (Huang et al, 1991). TD-OCT was relatively time consuming when compare to the newer OCT, as this technology used a mobile reference arm mirror to measure the light echo time delay sequentially. Therefore, it reduced the precise retinal tissue mapping due to the eye movement during the procedure. Hee et al (1995) have demonstrated the used of TD-OCT on human retina tissue with a high axial resolution of approximately 10 μm. It produces 400 axial scan per second.

1.5.2 Spectral Domain Optical Coherence Tomography

The OCT technology has evolved from the first generation TD-OCT to the second generation SD-OCT. SD-OCT used a broadband light source with approximately 830 nm together with a fixed reference arm mirror and a spectrometer, which significantly increase the scan speed to a new level of approximately 50,000 axial scan per second (Yaqoob et al, 2005). Other than this, SD-OCT also has a higher axial resolution of 5-7 μm (Wojtkowski et al, 2004; Ko et al, 2005), produced fewer artefacts (Forte et al, 2009) and improved signal-to-noise ratio (de Boer et al, 2003) as compare to the TD-OCT. Nowadays, numbers of SD-OCT are manufactured by different companies such as Cirrus HD-OCT by Carl Zeiss Meditec, 3D-OCT 1000 by Topcon Medical System, Spectralis OCT by Heidelberg Engineering GmbH, etc.

Since its advent, various studies have been conducted looking at the macular thickness, RNFL and ONH parameters among the subjects of different age, gender, race and risk factors such as diabetes, and myopia (Faghihi et al, 2010; Song et al, 2010). Furthermore, some of the newer SD-OCT have even further increases the accuracy in ONH parameter measurement. This is due to the ability of detecting the BMO, a terminus of the bruch's membrane. BMO is an important landmark to define the optic disc margin as well as to delineate the ONH from the peripapillary tissue in the OCT (Strouthidis et al, 2009). Besides, BMO also act as an reliable reference point as its location does not significantly change even in a diseased eye such as glaucoma; and there was no association between the location of BMO and age (Belghith et al, 2016). Deeper tissue scanning such as choroid thickness measurement is possible with the use of SD-OCT (Manjunath et al, 2010; Regatieri et al, 2012). However,

these measurements can still be limited due to the presence of RPE layer which can cause scattering and reduce penetration of the light.

Although the SD-OCT is far more superior to TD-OCT, the signal strength of an image is an important factor to decide the accuracy of a measurement. Cheung et al (2012) have demonstrated a significant difference in RNFL thickness between the images taken with signal strength of 10 and those with signal strength range from 5 to 7.

1.5.3 Swept Source Optical Coherence Tomography

SS-OCT is the third generation of OCT. In this latest technology, the narrowband swept laser source was chosen to produce a relatively longer wavelength, approximately 1050 nm. The longer wavelength gives the advantages on deeper tissue penetration including choroidal thickness measurement (Copete et al, 2014), less scattering of light, better axial resolution and more sensitive scan. Another plus point of SS-OCT is the high speed tissue scanning which is more than 100,000 scans per second (Potsaid et al, 2010).

1.6 RESEARCH FOCUS

Currently, there are very limited studies looking at the normative value of the ocular structure in β-thalassemia patients. This study will provide normative data of macular thickness, RNFL and ONH parameters in patients of HbE/ β-thalassemia which would serve as future reference and aid future researches. On the other hand, it may help to predict the changes of macular thickness, RNFL and ONH parameters in HbE/ β-thalassemia individuals.

Chapter 2

Objectives

STUDY OBJECTIVES

2.1 GENERAL OBJECTIVE

To evaluate the macular thickness, retinal nerve fiber layer (RNFL) thickness and optic nerve head (ONH) parameters in HbE/ beta thalassemia population.

2.2 SPECIFIC OBJECTIVES

2.2.1 To compare the mean macular thickness between the HbE/beta thalassemia and control.

2.2.2 To compare the mean RNFL thickness between the HbE/beta thalassemia and control.

2.2.3 To compare the ONH parameters between the HbE/beta thalassemia and control.

Chapter 3

Methodology

3.1 STUDY DESIGN

Cross sectional study.

3.2 STUDY POPULATION, SETTING AND PERIOD

The study was conducted from June 2014 until March 2016. Sixty-six HbE / β-Thalassemia patients, who were eligible, based on the selection criteria, were recruited from 3 centres; Ophthalmology Clinic in Hospital Sultanah Aminah (HSA), Hospital Raja Perempuan Zainab II (HRPZ II) and Hospital University Sains Malaysia (HUSM). The HbE/ β-Thalassemia patients and control subjects were matched according to age and gender.

3.3 SAMPLING AND SAMPLE SIZE

3.3.1 Sampling method

All patients who attended the Ophthalmology Clinic HSA, HRPZ II and HUSM between June 2014 and March 2016 and eligible based on the selection criteria, were included in the study. Convenience sampling was used for the HbE/ β-Thalassemia group. These patients were selected during their follow up in the Hematology Clinic. Simple random sampling was used for the control group which is from normal population.

3.3.2 Sample size

Sample size was calculated using Power and Sample Size Calculation (PS) Software version 3.0.10 using t-test formula.

3.3.2.1 Objective 1

To compare the macular thickness between HbE/ β-Thalassemia and the normal population.

- Mean macular thickness in HbE/ β-Thalassemia: no published data.
- Mean macular thickness in normal population: 275.66 ± 14.12um (Song et al, 2010).
- Sample: independent.
- Input: $\alpha = 0.05$, power =0.8, $\delta = 9$, $\sigma = 14.12$, m=1.
- Sample size: 40
- By the addition of 20% drop outs, the sample size that needs to be collected for each group is 48 subjects.

3.3.2.2 Objective 2

To compare the retinal nerve fiber layer (RNFL) thickness between HbE/ β-Thalassemia and the normal population.

- Mean RNFL in HbE/ β-Thalassemia: no published data.

- Mean RNFL in normal population.: 98.7 ± 10.9um (Seibold et al, 2010)
- Sample: independent.
- Input: α= 0.05, power =0.8, δ =6.0, σ = 10.9, m=1.
- Sample size: 53.
- By the addition of 20% drop outs, the sample size that needs to be collected for each group is 65 subjects.

3.3.2.3 Objective 3

To compare the optic nerve head (ONH) parameters between HbE/ β-Thalassemia and the normal population.

(i) To compare the mean disc area between HbE/ β-Thalassemia and the normal population.

- Mean optic disc area in HbE/ β-Thalassemia: no published data.
- Mean optic disc area in normal population: 1.98 ± 0.50 square millimetres (Resch et al, 2012).
- Sample: independent.
- Input: α= 0.05, power =0.8, δ =0.28, σ = 0.50, m=1.
- Sample size: 51.
- By the addition of 20% drop outs, the sample size that needs to be collected for each group is 60 subjects.

(ii) To compare the mean rim area (RA) between HbE/ β-Thalassemia and the normal population.

- Mean rim area in HbE/ β-Thalassemia: no published data.
- Mean rim area in normal population: 1.56 ± 0.49 square millimetres (Resch et al, 2012).
- Sample: independent.
- Input: α= 0.05, power =0.8, δ =0.28, σ = 0.49, m=1.
- Sample size: 49.
- By the addition of 20% drop outs, the sample size that needs to be collected for each group is 60 subjects.

(iii) To compare the mean cup volume (CV) between HbE/ β-Thalassemia and the normal population.

- Mean cup volume in HbE/ β-Thalassemia: no published data.
- Mean cup volume in normal population: 0.099 ± 0.11 cubic millimetres (Resch et al, 2012).
- Sample: independent.
- Input: α= 0.05, power =0.8, δ =0.06, σ = 0.11, m=1.
- Sample size: 54.
- By the addition of 20% drop outs, the sample size that needs to be collected for each group is 66 subjects.

(iv) To compare the mean average cup disc ratio (ACDR) between HbE/ β-Thalassemia and the normal population.

- Mean average cup disc ratio (ACDR) in HbE/ β-Thalassemia: no published data.
- Mean average cup disc ratio (ACDR) in normal population: 0.36 ± 0.17 (Resch et al, 2012).
- Sample: independent.
- Input: α= 0.05, power =0.8, δ =0.10, σ = 0.17, m=1.
- Sample size: 46.
- By the addition of 20% drop outs, the sample size that needs to be collected for each group is 56 subjects.

(v) To compare the mean vertical cup disc ratio (VCDR) between HbE/ β-Thalassemia and the normal population.

- Mean vertical cup disc ratio (VCDR) in HbE/ β-Thalassemia: no published data.
- Mean vertical cup disc ratio (VCDR) in normal population: 0.68 ± 0.14 (Moghimi et al, 2012).
- Sample: independent.
- Input: α= 0.05, power =0.8, δ =0.2, σ = 0.14, m=1.
- Sample size: 33.
- By the addition of 20% drop outs, the sample size that needs to be collected for each group is 40 subjects.

Based on objective 1, objective 2 and objective 3, the biggest sample size chosen for this study is 66 subjects for each group.

3.4 SELECTION CRITERIA

3.4.1 HbE/ β-Thalassemia Subjects

3.4.1.1 Inclusion criteria:

(i) Patients have been diagnosed with HbE/ β-Thalassemia and already in the National Thalassemia database.

3.4.1.2 Exclusion criteria:

(i) Previous ocular surgery and trauma.

(ii) Premorbid ocular pathology such as glaucoma, ocular hypertension, any macular or retinal pathology, any optic nerve pathology.

(iii) Myopia more than -3.00D.

(iv) Systemic co-morbidities such as diabetes mellitus, hypertension, and ischemic heart disease.

3.4.2 Control Subjects

3.4.2.1 Inclusion criteria:

(i) Healthy individuals.

(ii) Age matched.

3.4.2.2 Exclusion criteria

(i) Previous ocular surgery and trauma.

(ii) Premorbid ocular pathology such as glaucoma, ocular hypertension, any macular or retinal pathology, any optic nerve pathology.

(iii) Myopia more than -3.00D.

(iv) Systemic co-morbidities such as diabetes mellitus, hypertension, and ischemic heart disease.

3.5 ETHICAL APPROVAL

3.5.1 This study received ethical approval from Medical Research and Ethics Committee, Kementerian Kesihatan Malaysia on 17th December 2012 [Reg no: NMRR-12-980-13829] (Appendix A).

3.5.2 This study received ethical approval from Research and Ethical Committee, School of Medical Sciences, Universiti Sains Malaysia on 15th January 2013 [Reg No: 00007718; IRB Reg. No 00004494] (Appendix B).

3.6 FUNDING

This study, targeted genetic modifiers contributing to phenotypic heterogeneity of HbE/ β-Thalassemia and the psychological impact and health economic burden to the country, and was partially funded by the Research University Grant, Universiti Sains Malaysia (RU1001/PPSP/853003). (Appendix C).