A STUDY OF CAROTID INTIMA MEDIA THICKNESS AMONG THALASSEMIA PATIENT IN HUSM

By

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A STUDY OF CAROTID INTIMA MEDIA THICKNESS AMONG THALASSEMIA PATIENT IN HUSM

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Introduction: Thalassemia patient lifespan nowadays has been increased significantly compared previously due to the advancement of medical treatment and better healthcare system. As a result, more transfusion-related complication has been seen, and one of the rising trends was the thromboembolic complication. Studies had shown that ultrasound measurement of carotid artery intima media thickness (CIMT) can be used as a surrogate marker for future cardiovascular event and is recommended to be done in t halassemia patient as early diagnostic tool and for vascular risk stratification.

Objectives: The aim of this study were to compare the CIMT value between thalassemia patients and normal population and to find any correlation between thalassemia CIMT measurement with patient's age, disease duration, number of blood transfusion and serum ferritin levels.

Methods: All thalassemia patient attending treatment and follow-up at HUSM who fulfilled the inclusion criteria were recruited. An equal number of healthy subjects (gender matched) were taken as control group. Subjects from both groups with hypertension, diabetes mellitus, hypercholesterolemia, metabolic or connective tissue disease, on oral anti- coagulant, oral contraceptive pills, bed ridden and smoker were excluded. The CIMT measurement was performed by a single operator using an ultrasound machine (Siemen Acuson S2000) with 18 MHz linear array transducer. Both side of carotid arteries examination was performed with the subject in lying positioned and after a 10 minutes rest. An independent T-test was performed to compare mean. Regression analysis was used to look for association between CIMT and patient's age, disease duration, number of bood transfusion and serum ferittin level. All data analysis was performed using IBM SPSS software version 22.0 for Windows.

Results. A total of 80 subjects were included in this study with the equal number of thalassemia patients and the normal population. The mean value of CIMT of the general population was 0.32± 0.08mm and the mean value of thalassemia patient's CIMT was 0.45± 0.10mm. The independent t-test showed statistically significant differences of these measurement ($p<0.001$). On univariate analysis, there is a strong correlation between thalassemia CIMT measurement and disease duration and number of blood transfusions. However multivariate analysis showed only the number of blood transfusion is correlated with patient

CIMT measurement. Increased in the number of blood transfusion by 100 times will increase the mean CIMT by 1.0mm.

Conclusion: The CIMT measurement among thalassemia patient is significantly higher compared to general population and it is associated with the numbers of blood transfusion being received.

Dr Juhara Haron : Supervisor

AP Dr Ariffin Nasir : Co- supervisor

Dr Rosnah Bahar : Co- supervisor

Specially dedicated to:

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ABBREVIATIONS

AHA	American Heart Association
AT III	Antithrombin III
CIMT	Carotid intima-media thickness
CNS	Central nervous system
DVT	Deep vein thrombosis
eNOS	endothelial nitric oxide synthase
HUSM	Hospital Universiti Sains Malaysia
LDL	Low-density lipoprotein
NO	Nitrous oxide
ROS	Reactive oxygen species
SMC	Smooth muscle cells

ABSTRAK

Bahasa Malaysia

Tajuk: Kajian tentang ketebalan 'carotid intima-media' di kalangan pesakit thalassemia di HUSM.

Latarbelakang:

Jangka hayat pesakit thalassemia sekarang dapat dilanjutkan dengan adanya perkembangan pesat dalam teknologi perubatan dan prasarana kemudahan kesihatan. Akibatnya, timbul komplikasi- komplikasi baru dikalangan pesakit thalassemia ini dan diantara yang meningkat ialah komplikasi salur darah tersumbat (thromboembolism). Kajian menunjukkan ketebalan 'carotid intima-media'(CIM) yang diukur menggunakan mesin ultrasound dapat memberi gambaran dan stratifikasi tentang risiko serangan sakit jantung dan angin ahmar. Kajian terdahulu mensyorkan kajian CIM ini dilakukan keatas pesakit thalassemia sebagai alat diagnostik awal dan juga dapat mengetahui risiko untuk mendapat komplikasi salur darah tersumbat.

Objektif:

Bertujuan untuk mengetahui perbezaan ketebalan CIM diantara pesakit thalassemia dengan populasi bukan thalassemia. Kajian juga dilakukan untuk mengetahui hubungan diantara ketebalan CIM pesakit dengan umur, jangkamasa penyakit, bilangan transfusi darah dan kepekatan serum ferritin.

Kaedah:

Kajian telah dijalankan di Jabatan Radiologi, Hospital Universiti Sains Malaysia selama 19 bulan daripada Januari 2013 hingga Ogos 2014. Ketebalan CIM diukur oleh seorang penyelidik dengan menggunakan transduser selanjar 18MHz (Acuson S2000 Siemens). Semua pesakit thalassemia yang sedang menerima rawatan atau rawatan susulan di HUSM dan bersetuju untuk mengikuti kajian ini telah diambil untuk kajian ini. Selain itu umur pesakit, jangkamasa penyakit, bilangan transfusi darah dan paras serum ferritin pesakit thalassemia juga diambil dan direkodkan.

Keputusan:

Seramai 80 subjek terlibat dalam kajian ini dimana jumlah subjek thalassemia dan bukan thalassemia adalah sama. Nilai purata ketebalan CIM pesakit thalassemia adalah 0.45± 0.1mm dan nilai purata ketebalan CIM populasi bukan thalassemia adalah 0.32± 0.08mm. Terdapat perbezaan yang signifikan secara statistik diantara nilai purata ketebalan CIM pesakit thalassemia dengan populasi bukan thalassemia. Kajian juga mendapati terdapat hubungkait yang signifikan diantara bilangan transfusi darah dengan ketebalan CIM pesakit thalassemia. Kenaikan 100 kali bilangan transfusi darah akan mengakibatkan kenaikan ketebalam CIM pesakit thalassemia sebanyak 1.0mm.

Kesimpulan:

Berdasarkan keputusan ketebalan CIM yang telah dibuat, pesakit thalassemia mungkin berisiko lebih tinggi berbanding dengan populasi bukan thalassemia untuk mendapat serangan jantung dan angin ahmar dan ianya berkait rapat dengan bilangan transfusi darah pesakit.

ABSTRACT

English

Title: A study of carotid intima-media thickness among thalassemia patients in HUSM.

Background:

Thalassemia patient lifespan nowadays has been increased significantly compared to previously due to the advancement of medical treatment and better healthcare system. As a result, more transfusion-related complications are seen, and one of the rising trends is the thromboembolic complication. Studies has shown that ultrasound measurement of carotid intima-media thickness (CIMT) can be used as a surrogate marker for future cardiovascular event and is recommended to be done in thalassemia patient as early diagnostic tool and for vascular risk stratification.

Objectives:

To compare CIMT measurement between thalassemia patients in HUSM with the non- thalassemia population and to find any association between CIMT measurement with patient's age, disease duration, numbers of blood transfusions and serum ferritin level.

Methodology:

A cross sectional study was done over a period of 19 months from January 2013 until August 2014. A single operator performed the ultrasound examination using 18 Mhz

linear array transducer (Siemen Acuson S2000) at Department of Radiology, Hospital Universiti Sains Malaysia (HUSM). All thalassemia patient who is receiving treatment and follow-up at HUSM and consented for the examination, were subjected to the measurement of their carotid intima-media thickness (CIMT). Patient age, disease duration, numbers of blood transfusion and serum ferritin level were obtained and recorded. The same numbers of healthy subject were recruited from the general population and their CIMT were also recorded.

Result:

A total of 80 subjects were included in this study with the equal number of thalassemia patients and the non- thalassemia population. The mean value of CIMT for the non- thalassemia population was 0.32± 0.08mm and that of thalassemia patient was 0.45± 0.10mm. Independent t-test showed statistically significant difference between these two measurements ($p<0.001$). On univariate analysis, there was a strong correlation between thalassemia CIMT measurement and disease duration and number of blood transfusions. However multivariate analysis showed only the number of blood transfusion was significantly correlated with patient CIMT measurement. Increased in the number of blood transfusion by 100 times would increase the mean CIMT by 1.0mm.

Conclusion:

The finding of higher mean value of CIMT in thalassemia patient might be suggestive for an increased in future cardiovascular and cerebrovascular event in thalassemia patient compared to non- thalassemia population and it is significantly associated with the number of blood transfusion.

1.0 INTRODUCTION

Thalassemia is classified as a group of congenital hereditary blood disorders in which the anomalies are within the synthesis of the chains of haemoglobin. The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world and in Malaysia, it is estimated about 4.5% of its population are heterozygous carriers for beta- thalassemia and the couples are at risk of having beta thalassemic child about 2.1/1000 births annually (George, 2001). Clinical presentations of thalassemia range from totally asymptomatic individuals to severe anaemia which needs regular blood transfusion. It is estimated that about 4,800 thalassemia major patients in Malaysia which need regular blood transfusion (Elizabeth and Ann, 2011).

Common problem encountered by thalassemia major patient from their chronic anaemic state is tissue iron deposition as a result from the frequent blood transfusion and increased in gastrointestineal iron absorption. As a consequence, toxic iron will accumulate within liver, heart, spleen and endocrine organs. Chelation therapy had been commenced to counter iron overload in thalassemia patient and desferoxamine mesylate has been the standard treatment for iron chelation therapy for decades (Delea *et al.*, 2007). Although current thalassemia patients' survival has increased due to chelation therapy, cardiovascular complications are still common (Hahalis *et al.*, 2008) and about 70% of all thalassemic deaths are due to heart failure

and arrhythmias (Borgna-Pignatti *et al.*, 2004). This may be due to issue of compliancy because of the discomfort during the iron chelating therapy administration and due to its high cost (Delea *et al.*, 2007; Dahlui *et al.*, 2009; Viprakasit *et al.*, 2009). The compliancy of desferoxamine usage had been shown to reduce the serum ferritin level as well as cardiac complication (Wolfe *et al.*, 1985).

Besides that, incidence of vascular complications has been reported in thalassemia patients, mainly attributed by the hypercoagulable state and vascular dysfunction (Hahalis *et al.*, 2008). Atherosclerosis is a formation of an atherosclerotic plaque within the arterial lumen. It usually started as lipid-filed macrophages or foam cells in the early phase which can be replaced by collagen fibres in later stage. As the size of the atherosclerotic plaque increases, it can cause narrowing of the vessel lumen. The thickening of the luminal wall can be detected by several imaging modalities especially the intima-media complex which also been called as carotid intima media thickness (CIMT).

CIMT can be measured by using ultrasound, computed tomography (CT) scan or magnetic resonance imaging (MRI). Ultrasound examination is by far the most preferred technique as it is non-radiating and relatively inexpensive. CT scan measurement is highly reproducible compared to ultrasound and its measurement can be considered as tomographic equivalent of sonographic CIMT (Saba *et al.*, 2008). However the radiation hazard from the examination limits its daily usage. MRI

examination is another examination which is potential to have higher reproducibility (Underhill *et al.,* 2006). However the availability is usually limited compared to ultrasound and the cost is much higher.

Several studies have shown that sonographic carotid intima media thickness (CIMT) measurement is increased in thalassemia patients indicating premature atherosclerosis. The same studies also shown that sonographic CIMT measurement in thalassemic patients' are correlated with patients' age (Tantawy *et al.*, 2009), disease duration (Dogan and Citak, 2011) and serum ferritin level (Tantawy *et al.*, 2009; Ismail and El-Sherif, 2010; Dogan and Citak, 2011). Sonographic CIMT measurement, which is a recognised surrogate marker for future cardiovascular events (Bots *et al.*, 1997) is recommended to be done in beta thalassemia patients as a non-invasive early diagnostic tool (Ismail and El-Sherif, 2010; Dogan and Citak, 2011) and for vascular risk stratification (Tantawy *et al.*, 2009).

There are few sonographic CIMT measurement studies done on thalassemia patients worldwide however there is no similar study has yet to be done in Southeast Asia, particularly Malaysia which mainly comprised of HbE β-thalassemia, which is a structural β-globin Hb variant with a β+ phenotype (George, 2013). HbE β-thalassemia shows highest frequencies in Asia such as India, Bangladesh, Thailand, Laos, Cambodia (Olivieri *et al.*, 2011) and also in Malaysians' Malay population (George, 2013). By doing this study, it is hoped that the local thalassemia CIMT

measurement can be measured and compared with the normal population. Any difference in the mean and standard deviation will be analyzed and any increment in the CIMT would indicate subclinical atherosclerosis and increased risk of having future thrombosis event. If there is evidence of subclinical atherosclerosis in the local thalassemia population, than perhaps there would be a role of anti-thrombolytic agent in thalassemia patient in the future. Any significant correlation between patient CIMT with patient's age, disease duration, number of blood transfusion and serum ferritin level would greatly help us to understand more regarding this new emerging complication finding.

2.0 LITERATURE REVIEW

2.1 Anatomy of the common carotid artery

Both right and left common carotid arteries differ in length and in their mode of origin. There are many variants besides the common pattern. The knowledge of the normal common carotid artery anatomy and its variants are important during the ultrasound assessment.

2.1.1 Vascular anatomy

The brain received its blood supply mainly from 4 main vessels, right and left internal carotid arteries and right and left vertebral arteries. The internal carotid artery derived from common carotid artery on each side of the neck. The right common carotid artery derives from the brachiocephalic trunk which is the first branch of arch of aorta while the left common carotid artery derived directly from aortic arch in the superior mediastinum (Figure 2.1). The right common carotid artery has cervical part while left common carotid artery has cervical and thoracic part (Standring, 2008).

The brachiocephalic trunk travels superiorly, slightly posterior from the aortic arch to the right of the neck for about 4 to 5cm in length before dividing into right common carotid artery and right subclavian artery at the upper border of right sternoclavicular junction (Zwiebel, 2000). The left common carotid artery travels upwards from the aortic arch and passes beneath left sternoclavicular joint. Both common carotid arteries then divides into internal and external carotid arteries at the

level of upper border of thyroid cartilage (Zwiebel, 2000). Both common carotid arteries do not give collateral branches.

2.1.2 Normal variants.

There are many normal variants of the origin of these vessels (Butler *et al.*, 2012). The most common appearance of the aortic arch and it normal variations are shown in figure 2.1 and figure 2.2. The knowledge of the normal variants is important because it might cause difficulties in identifying the respective vessels if present.

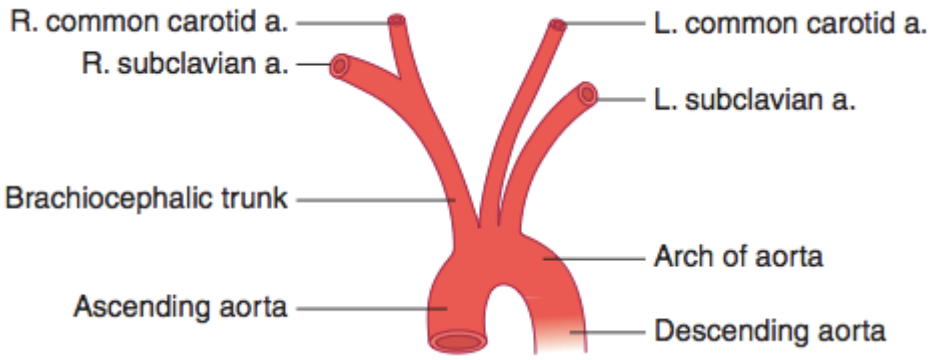


Figure 2.1: Moss common appearance of aortic arch. (Adapted from Butler et al., 2012)

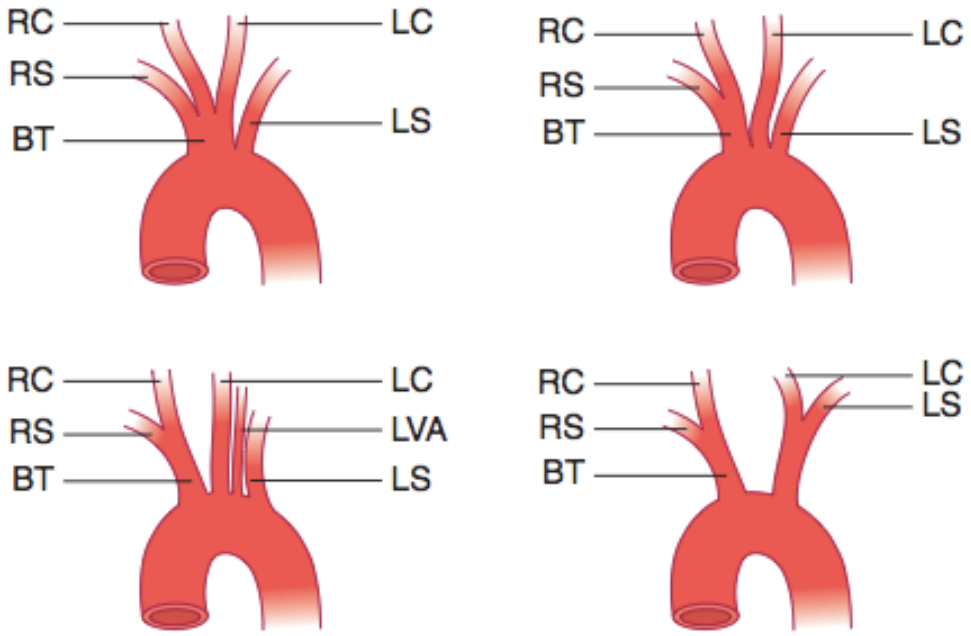


Figure 2.2: Normal variant of the aortic arch and origin of the common carotid artery. Key: RS- right subclavian artery, RC- right common carotid artery, BT – brachiocephalic trunk LC- left common carotid artery, LS- left subclavian artery and LVA- left vertebral artery. (Adapted from Butler et al., 2012)

2.1.3 Normal arterial wall structure

Traditionally, based on the basis of size and characteristics of tunica media, there are 3 different types of arteries (Pawlina and Ross, 2011) which are the large arteries, medium arteries and small arteries or arterioloes. Common carotid artery is categorized under large artery and has 3 distinct layers which are the intima or the epithelial lining of the artery, media or muscular layer and adventitia. The intima is the innermost layer, followed by media which is the middle layer and adventitia, the outermost layer (Figure 2.3).

The tunica intima consist mainly of three component; the endothelium which is a single layer of squamous epithelial cell; a thin layer of extracellular cell called basal lamina which composed of collagen, proteoglycan and glycoprotein; and lastly the subendothelial layer which consists of loose connective tissue. Smooth muscle cell occasionally can be found within the loose connective tissue. This subendothelial layer contained sheet like layer or lamella of fenestrated elastic material called the internal elastic membrane. These fenestrations enable substances to diffuse readily through the layer and reach cells deep within the wall of the vessel.

The tunica media, or middle layer, consists primarily of circumferentially arranged layers of smooth muscle cells (Standring, 2008). This layer has variable amounts of elastin, reticular fibers, and proteoglycans which is interposed between the smooth muscle cells of the tunica media. It extends from the internal elastic membrane to external elastic membrane and is relatively thick. The external elastic

membrane is a layer of elastin that separates the tunica media from the tunica adventitia. The sheets or lamellae of elastin are fenestrated and arranged in circular concentric layers.

The tunica adventitia, or outermost connective tissue layer, is composed primarily of longitudinally arranged collagenous tissue and a few elastic fibers. It will gradually merge with the loose connective tissue surrounding the vessels. This tunica adventitia is relatively thin. Most tunica adventitia layers of large arteries contains a system of vessels that supplies blood to the vascular walls themselves called the vasa vasorum, as well as a network of autonomic nerves called nervi vascularis that control contraction of the smooth muscle in the vessel walls. The main cell in the adventitia layer is the smooth muscle cells. Their main function is for structural support of the artery. They regulate the size of the arterial lumen and hence the blood flow and blood pressure by their contractility responses. The smooth muscle cells also responsible for synthesis of all constituents of the arterial wall. It is also capable of endocytosis of foreign materials and lipoproteins. Besides that, the smooth muscle cells also produce all of the extracellular components of the tunica media. The function of smooth muscle cells are summarize in table 2.1.

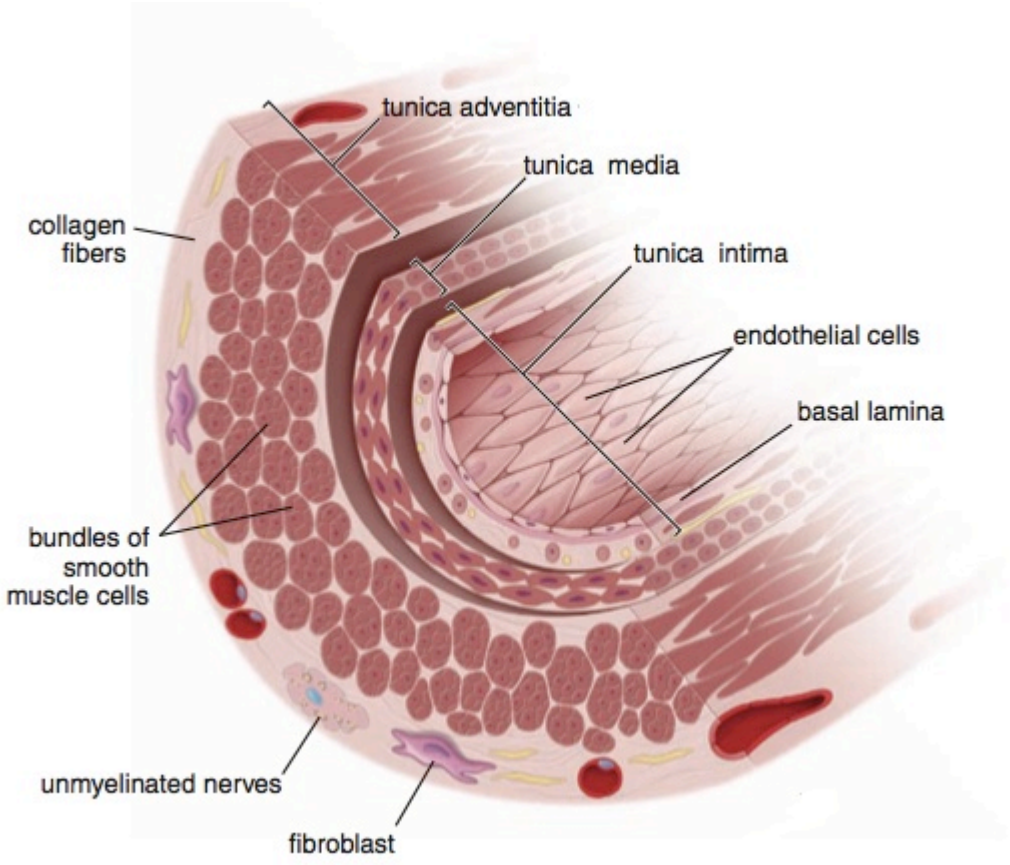


Figure 2.3: Normal arterial wall structure. (Adapted from Pawlina and Ross, 2011)

Table 2.1: Functions of arterial smooth muscle cells

1. Structural support
2. Contractile response
3. Synthetic/ metabolic/ secretory function - Actin - Myosin - Collagen - Elastin - Microfibrillar proteins - Proteoglycans - Lipids
4. Endocytosis

2.1.4 Normal vascular endothelium layer structure and functions

The endothelium layer is formed by a continuous layer of flattened, elongated, and polygonally shaped endothelial cells that are aligned with their long axes in the direction of the blood flow. At the luminal surface, they express a variety of surface adhesion molecules and receptors such as low-density lipoprotein, insulin, and histamine receptors. Endothelial cells have many functions (Table 2.2). It plays an important role in blood homeostasis in which these cells can change their functional properties in response to various stimuli. This process, known as endothelial activation, is also responsible for the pathogenesis of many vascular diseases particularly atherosclerosis. Inducers of endothelial activation include bacterial and viral antigens, cytotoxins, complement products, lipid products, and hypoxia. Activated endothelial cells exhibit new surface adhesion molecules and produce

different classes of cytokines, lymphokines, growth factors, and vasoconstrictor and vasodilator molecules, as well as molecules that control blood coagulation. Endothelial cells also participate in the structural and functional integrity of the vascular wall.

Apart from that, endothelial cells are also active participants in a variety of interactions between the blood and underlying connective tissue and are responsible for many properties of the vessels. First, endothelial cells exhibit a selective permeability barrier which allows selective movement of small and large molecules from the blood to the tissues and from the tissues to the blood. This movement is related to the size and charge of the molecules. Through a process called simple diffusion, small hydrophobic (lipid-soluble) molecules such as oxygen or carbon dioxide can readily pass through the permeable lipid bilayer of the endothelial cell membrane. However, water and hydrophilic (water-soluble) molecules such as glucose, amino acids and electrolytes cannot diffuse across the endothelial cell membrane and these molecules and solutes have to be actively transported. Secondly, the endothelial layer functions as a nonthrombogenic barrier between blood platelets and subendothelial tissue which is done by producing anticoagulants and antithrombogenic substances. Normal endothelium does not support the adherence of platelets or the formation of thrombi on its surface. However, damaged endothelial cells cause them to release prothrombogenic agents such as von Willebrand factor or plasminogen-activator inhibitor to promote thrombus formation. The modulation of

blood flow and vascular resistance is achieved by the secretion of vasoconstrictors such as endothelins, angiotensin-converting enzyme, prostaglandin, thromboxane and vasodilators such as nitrous oxide (NO).

The contraction and relaxation of smooth muscle cells in the tunica media influencing local blood flow and pressure also is being controlled by the endothelium layer. Shear stress produced during the interaction of blood flow with vascular endothelial cells initiates nitric oxide-derived relaxation of blood vessels. Endothelium-derived nitric oxide is one of several critical regulators of cardiovascular homeostasis. It regulates the blood vessel diameter, inhibits monocyte adhesion to dysfunctional endothelial cells, and maintains an antiproliferative and anti-apoptotic environment in the vessel wall. Nitric oxide is an endogenous vasodilatory gas which continuously being synthesized in endothelial cells by endothelial nitric oxide synthase (eNOS). It acts as an anti-inflammatory agent under normal physiologic conditions, although its overproduction induces inflammation. Nitric oxide is also involved in immune reactions, a potent neurotransmitter in the nervous system, and also contributes to the regulation of apoptosis.

Other function of endothelial cell includes regulation and modulation of the immune responses. It also synthesizes, metabolizes and secretes many substances,

such as prostacyclin, angiotensin-converting enzyme, clotting factor VIII and lipoprotein lipase.

Table 2.2: Functions of endothelial cells

1. Blood compatible container
2. Selective permeability barrier
3. Synthetic/ metabolic/ secretory function - Angiotensin-converting enzyme - Factor VII - Plasminogen - Von-Willebrand factor - Prostacyclin - Thromboxane - Fibronectin - Collagen (type IV) - A-2-macroglobulin - Lipoprotein lipase - Hormone receptors
4. Binding and internalization of lipoproteins

2.2 Atherosclerosis

Atherosclerosis is the process of atherosclerotic plaque formation within the arterial lumen. Atherosclerotic plaques can be characterized by tunica intimal thickening due to progressive accumulation of lipids together with numerous cellular and molecular components such as smooth muscle cells (SMC), lipid-filled macrophages, monocytes, T and B lymphocytes, erythrocytes, and platelets (Nicolaides *et al.*, 2011). American Heart Association (AHA) Committee on Vascular Lesion has divided plaques into six stages according to the plaque composition and morphology based on histologic studies of human vessels, mainly coronary and aortic arteries obtained at autopsy.

2.2.1 Early Lesions (Types I and II)

These plaques appear during the first decades of life and usually do not cause substantial luminal stenosis. In type I plaques, the histological changes are minimal, which consist of isolated groups of lipid-filled macrophages or foam cells that are visible only with microscopic examination (Figure 2.4). These are in contrast to type II plaques or fatty streaks which are visible on gross examination and contain increased numbers of foamy macrophages. It will become stratified into layers together with some foamy smooth muscle cells (Figure 2.4). The main source of plaque lipids is the circulating LDL particles that become entrapped within the subendothelial layer as evidence by the strong similarity between the chemical

composition of the low-density lipoprotein (LDL) particles and plaque lipids. The process in which LDL particles can appear within the vessel wall is either by passive diffusion through the endothelium or by receptor-mediated endocytosis. Subsequently, through ionic interactions between the apolipoprotein-B of the LDL particle and matrix proteoglycans, collagen fibers, and fibronectin found in the vessel wall, retention of LDL particles within the vessel wall will occur. The trapped LDL particles will then undergo extensive modifications such as oxidation, proteolysis, aggregation, and lipolysis. Minimally oxidized LDL particles (mmLDL) are recognized by the LDL receptor, and their accumulation will stimulate endothelial cells to promote recruitment of monocytes and lymphocytes to the vessel wall. Severely oxidized LDL particles in contrast, can only be recognized by scavenger receptors that are expressed on macrophages and vascular smooth muscle cells. The uptake of oxidized LDL will cause formation of foam cells (Figure 2.4).

2.2.2 Preatheroma/Intermediate Lesions (Type III)

Type III plaques have a histological appearance that is in between the early fatty streaks and the first advanced lesion type or atheroma. However, it is not known how the plaques progress from one stage to the other, whether it progress linearly or not. In type III plaques, organized histological layers can be seen. Foamy cells are present at the luminal side with tissue degeneration region seen in the middle layer and scattered extracellular lipids noted at the base of the plaque (Figure 2.4). Type III plaques contain more free cholesterol, fatty acids, triglycerides, sphingomyelin, and

lysolecithin than type II plaques. An intermediate type of plaque can directly transform into an advanced and more complicated lesion.

2.2.3 Advanced Atherosclerotic Plaques (Atheroma-IV, Fibroatheroma-Va, Calcific-Vb and Fibrotic-Vc)

In type IV plaque or atheroma, there is abundant accumulation of extracellular lipids is seen. The lipids form a consolidated core located deeply within the intima and disorganizes the extracellular matrix. The region of the thickened intima between the lipid core and the endothelial surface contains smooth muscle cells, macrophages with and without lipid droplets, T lymphocytes and mast cells (Figure 2.4). Proteoglycan will be secreted by the smooth muscle cells in and few collagen fibers may gradually thicken at the region above the lipid core. At the base of the atheroma, cell death and formation of a necrotic core will occurs which is rich in cellular debris and crystalline cholesterol. Atheroma usually do not cause severe luminal narrowing, however there are susceptible to fissure formation and ruptures to become a complicated plaque due to their surface composition.

Fibroatheromas or type Va plaques have a thick layer of fibrous connective tissue. It mainly contains collagen fibers and rough endoplasmic reticulum–rich smooth muscle cells at the luminal side of the intima which is called the fibrous cap. It separates the lipid core from circulating blood constituents. The capillaries at the

borders of the lipid core may be larger and more numerous compared to those found in type IV plaques.

Type Vb plaques or calcified plaques is characterized by increased in its mineralization. In type Vc or fibrotic lesions, the intima thickening is primarily due to accumulation of collagen fibers instead of lipid accumulation. Type V plaque can suddenly transform to type VI plaque with formation of surface defects like erosions, fissures and ruptures or with the formation of hematomas.

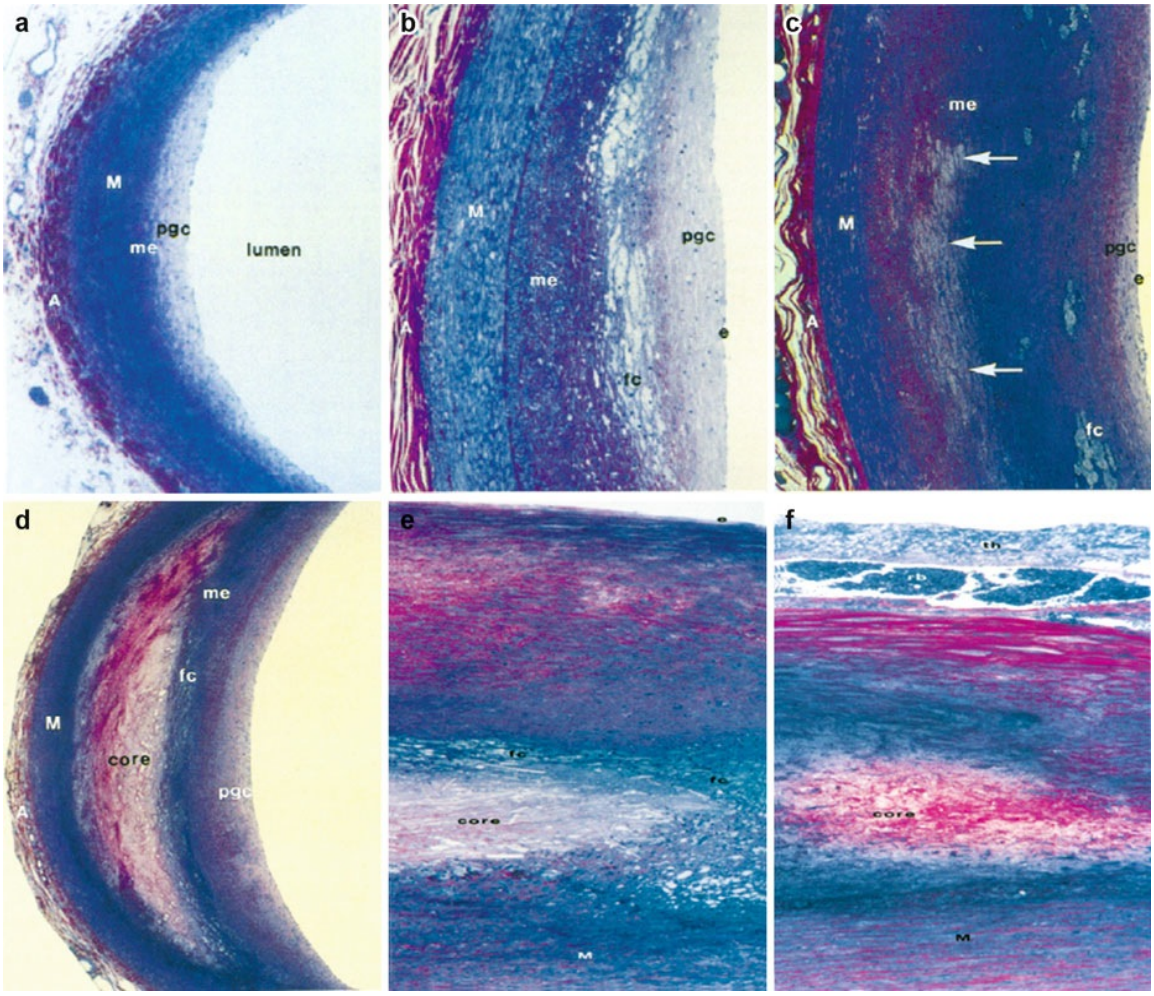


Figure 2.4: Histological examples of atherosclerotic plaque types classified according to the American Heart Association criteria (Adapted from Nicolaides *et al.*, 2011).

(a) A crescent-shaped type I intimal thickening. Pgc=proteoglycan intima layer, me= musculoelastic intima layer, M=media, A=adventitia, lumen=lumen of the artery

(b) A type II (progression-prone fatty streak) lesion. Macrophage foam cells (fc) occupy the intima at the junction of the proteoglycan (pgc) and musculoelastic (me) intima layers, e=endothelial cells at the artery lumen, M=media, A=adventitia.

(c) A type III (preatheroma) lesion. Extracellular lipid (arrows) is pooled in the musculoelastic layer (me). Smooth muscle cells, normally closely packed, are separated, compressed, and attenuated by the extracellular lipid. Macrophage foam

cells (fc) are some distance above the pooled extracellular lipid, endothelial cells (e) at the artery lumen, pgc= proteoglycan intima, M= media, A= adventitia.

(d) A type IV (atheroma) lesion. In addition to all the changes seen in type IIa and III lesions, a massive aggregate of extracellular lipid (lipid core) occupies the musculoelastic layer (me). Macrophage foam cells (fc) are above the lipid core. Pgc= proteoglycan intima layer, M=media, A=adventitia

(e) A type V (fibroatheroma) lesion in the distal part of the abdominal aorta. The part of the lesion above the lipid core and above the layer of macrophage foam cells (fc) consists of dense bands of collagen, endothelial cells (e) at the artery lumen, M= media,

(f) A type VI (complicated) lesion in the distal recruited through the activated endothelium differentiate into macrophages. Several endogenous and microbial molecules can ligate pattern-recognition receptors on these cells, inducing activation and leading to the release of inflammatory cytokines, chemokines, oxygen and nitrogen radicals, and other inflammatory molecules and, ultimately, to inflammation and tissue damage

2.3 Thalassemia

Thalassemia syndromes are inherited disorders due to abnormal α or β-globin biosynthesis. The reduce supply of globin will reduce production of haemoglobin tetramers, leading to hypochromia and microcytosis. The synthesis of the unaffected globins proceeds at normal rate will cause unbalanced accumulation of alpha or beta subunits. Normally all four alpha genes and both beta genes are active in the production of globin chains. In beta thalassemia, synthesis of the beta chain is defective where as in alpha thalassemia, the synthesis of the alpha chain that is defective. Beta thalassemia is common in the Mediterranean region and in portions of Africa, Asia, the South Pacific, and India while alpha thalassemia is most common in Southeast Asia. Thalassemia patients have a wide spectrum of clinical presentations, ranging from totally asymptomatic to severe anaemia which need regular blood transfusion. This depends on the degree to which the synthesis of the affected globin is impaired, altered synthesis of other globin chains and coinheritance of other abnormal globin alleles (Longo, 2013).

In beta thalassemia, point mutations or a partial deletion of chromosome 11 cause defective synthesis of the beta chain. Over 100 mutations have been identified. Normally alpha and beta globin chains are made approximately in equal amounts. When beta globin chains are in short supply or absent, as in beta thalassemia, alpha chains are in excess. The excess alpha chains combine with other available beta family globin chains (delta or gamma) to form increased amounts of Hgb A2 ($\alpha 2\ \delta 2$) and Hgb F ($\alpha 2\ \gamma 2$). Hgb Barts ($\gamma 4$) or tetramers of excess gamma chains may also

form (Figure 2.5). Because of the reduced amounts of haemoglobin tetramers, all beta thalassemia haemoglobin are characterised by hypochromic and microcystosis. In heterozygotes or known as beta thalassemia trait, this is the only abnormality seen and the anaemia is usually minimal. In more severe form of homozygous state, there will be highly insoluble unpaired alpha chains due to unbalanced alpha and beta-globin accumulation. This in turn will form toxic inclusion bodies that kill developing erythroblast in the marrow. Few erythroblasts will survive and mature and it will then bear a burden of inclusion bodies that are detected in the spleen. The red blood cell life span will be reduced leading to haemolytic anaemia. Marrows will response with increased production of erythroblast however the anaemia will still persist due to ineffective erythropoiesis. Based from the abnormal genetic mutation inherited and clinical syndromes, four phenotypes of beta-thalassemia had been categorised: beta-thalassemia carrier, beta-thalassemia trait, beta-thalassemia intermedia and beta-thalassemia major. Beta-thalassemia carrier and trait are usually asymptomatic, whereas beta-thalassemia major needs frequent blood transfusion. Unlike thalassemia major, beta-thalassemia intermedia patients usually does not need frequent blood transfusions and commonly presented later, during their early adulthood period.

One to four alpha genes may be deleted in alpha thalassemia disorders. The clinical manifestations of alpha thalassemia vary with the number of alpha-chain genes that are deleted from chromosome 16. If only one alpha gene is deleted, no hematologic abnormalities are seen. This is known as a silent carrier state. If two alpha genes are deleted, either homozygous (a-/a-) or heterozygous (--/aa), the

condition is alpha thalassemia trait. The heterozygous type is encountered in Southeast Asian populations, but is rare in Afro-Americans. Alpha thalassemia trait results in microcytosis, hypochromia, and mild anaemia. If three alpha genes are deleted (--/-a), there will be an accumulation of unpaired beta chains and are soluble enough to form β4 tetramers called hemoglobin H (HbH) (Figure 2.6). Patient with hemoglobin H disease usually will behave like thalassemia intermedia, characterised by moderately severe haemolytic anaemia but milder ineffective erythropoiesis. It also common for the patient to survive to mid-adult life without needing blood transfusion. If all four of the alpha genes are deleted, it is called hemoglobin Barts which is incompatible with life and usually results death in utero. The red blood cells contain only Bart's haemoglobin, a tetramer of gamma chains which is incompatible with life. This condition which also known as hydrops fetalis is usually encountered in Asian and African population.

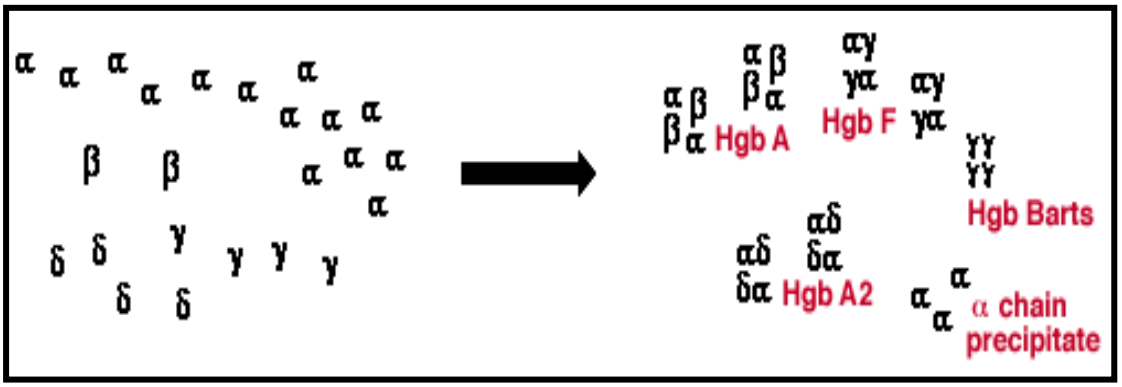


Figure 2.5: Type of beta thalassemia. (Source: http://www.med-ed.virginia.edu/courses/path/innes/rcd/thalassemia.cfm)

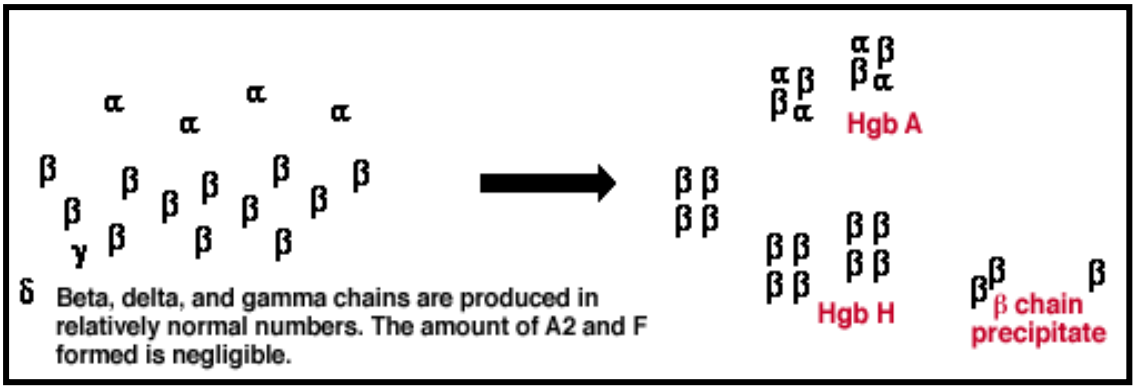


Figure 2.6: Type of alpha thalassemia. (Source: http://www.med-ed.virginia.edu/courses/path/innes/rcd/thalassemia.cfm)

2.4 Hypercoagulability State in Thalassemia Patient

Due to the better health care support, current thalassemia patient lives longer compared previously. As a result, new complication were identified in this type of patient and the commonest problem that had been increasingly recognised is thromboembolic events (Taher *et al.*, 2008) (Panigrahi and Agarwal, 2007). An Italian multicentre study (Borgna Pignatti *et al.*, 1998) revealed that 27 of 683 (3.95%) thalassemia major and 5 of 52 (9.61%) thalassemia intermedia patient developed thromboembolic events. About half the events involved central nerve system (CNS) location, followed by deep vein thrombosis (DVT) (25%), pulmonary (9.3%), portal (6.3%) and intracardiac (6.3%). Another study done in Mediterranean regions and Iran (Taher *et al.*, 2006) showed that 146 out of 8860 (1.65%) thalassemia patient had experienced thromboembolic event, 61 with thalassemia major (0.9%) and 85 with thalassemia intermedia (3.9%). According to the study, the highest occurrence of thromboembolic events that occur in thalassemia were deep vein thrombosis (32%),

followed by stroke (18%), portal vein thrombosis (16%), pulmonary embolism (13%) and superficial thrombophlebitis (4.7%). The study also noted that venous thrombosis was more common in thalassemia intermedia while arterial events were significantly more in thalassemia major patients. Thrombosis was noted more common in female, splenectomised patients and those with profound anemia.

There are many factors contributing to the hypercoagulable state in thalassemia patients (Table 2.3). The first factor is platelet abnormalities. Many studies have showed that there is impairment of platelet aggregation, increased in circulating platelet aggregates and shortened platelet lifespan (Eldor and Rachmilewitz, 2002; Panigrahi and Agarwal, 2007; Taher *et al.*, 2008; Cappellini *et al.*, 2010). There is also chronic platelet activation in patient with thalassemia, which is more pronounced in patient with beta thalassemia undergoing splenectomy.

Apart from that, there are defects in coagulation inhibitors and plasma markers in thalassemia major patients. Irrespective of the patient's age, the coagulation inhibitors-protein C (PC) and protein S (PS) are significantly decreased in patients with thalassemia major. Study done among thalassemia major patients in Hospital Universiti Sains Malaysia also showed similar result when compared to normal blood donors (Rosnah *et al.*, 2014). Some thalassemic patient from Italy and Turkey had low antithrombin III (AT III) in addition to protein C and protein S deficiency. Heparin co-factor II (HC II) which is another coagulation inhibitor, is also low in thalassemia patients, and this is known to be associated with increased thrombotic risk.

Red blood cells also play roles in the hypercoagulability state in thalassemia patient. The oxidation of globin subunits in thalassemia erythroid cells will alter the red blood cells leading to the formation of hemichromes. Hemichromes bind to or modify various components of the mature red blood cell membrane. Heme will disintegrate after the precipitation of hemichromes, and toxic nontransferrin-bound iron species are released from the heme disintegration. The free iron will then catalyzes the formation of reactive oxygen species which in turn cause thalassemic red cells to become rigid and deformed and to aggregate, resulting in premature cell destruction. Studies also have shown that thalassemic red blood cells may be a source of negatively charged phospholipids (Eldor and Rachmilewitz, 2002; Panigrahi and Agarwal, 2007; Taher *et al.*, 2008; Cappellini *et al.*, 2010), which can eventually increase thrombin generation. Several studies have also demonstrated that RBCs from thalassemic patients show enhanced cohesiveness and aggregability which will be reduced to normal range after the patients have received a blood transfusion.

Due to increase in haemolysis process, plasma iron and hemin levels are elevated in beta thalassemia patients. This will lead to generation of labile iron at the inner and outer cell surfaces, exposing the cell to conditions whereby the labile metal promotes the formation of reactive oxygen species (ROS). This will lead to cumulative cell damage. Higher ROS levels were seen in patient with beta thalassemia compared to normal blood donors suggesting that there is a chronic oxidative stress in patients with β-thalassemia. This will leads to platelet activation and susceptibility to thromboembolic consequences.

Thalassemia patients also have inherited prothrombotic mutations such as heterozygosity for Factor V Leiden, MTHFR C677T homozygosity and prothrombin G20210A mutation leading to hypercoagulability state. Other contributing factors for thromboembolic events are associated abnormalities like dilated cardiomyopthy, hypothyroidism and hyperglycemia.

Table 2.3: Causes of hypercoagulable state in thalassemia patient

1. Red blood cells - Abnormal erythoid cells - Source of procoagulant phospholipids - Enhanced cohesiveness
2. Platelets - Increased platelet aggregations - State of oxidative stress - Expression of activation markers
3. Abnormal coagulation profile - Decreased Protein C, Protein S and anti-thrombin III
4. Iron overload
5. Endothelial damage
6. Hyperviscosity
7. Genetic mutations - Factor V Leiden - Prothrombin
8. Others - Cardiac dysfunction - Liver dysfunction - Hormonal deficiencies - Antiphospholipid antibodies

2.5 Radiological Investigation of Carotid Diseases.

There are many radiological carotid diseases imaging techniques which include ultrasound, computed tomography angiography (CTA), MRI and digital subtraction angiography (DSA). Ultrasound is by far the most widely employed imaging modality and the screening method of choice because it is relatively inexpensive, portable, and completely non-invasive, it can be repeated as often as necessary, and it has good diagnostic accuracy for the detection of significant stenosis. However its evaluation is restricted to a limited portion of the carotid artery, image quality is not always optimal, and reproducibility is limited because of operator- dependent factors. Thus, there is substantial variability in the performance of the test amongst different centres (Jahromi *et al.*, 2005).

DSA has been the confirmatory imaging modality before surgical intervention for many years, and still it is considered as the reference standard for the detection and quantification of carotid stenosis until today. However it is invasive in nature with possible of neurological and vascular complications (Willinsky *et al.*, 2003). DSA also involved in using ionizing radiation and nephrotoxic contrast agents which may be limited in patients with renal disease, and it is expensive and relatively lengthy.

CTA is an alternative to DSA which is less invasive. With the newer generations of multidetector spiral scanners, CTA can provide complete three-dimensional (3D) coverage of the carotid tree which is highly accurate in detecting

luminal stenosis (Koelemay *et al.*, 2004). Study also showed that carotid artery wall thickness can be measured by this method and can be considered the tomographic equivalent of CIMT measured by ultrasound (Saba *et al.*, 2008) However it also have its limitation due to radiation exposure and risk of contrast-induced nephrotoxicity.

MRI has emerged as the most widely employed non-invasive modality if a confirmatory test is needed after ultrasound examination. MRI does not involve in ionizing radiation and there is less significant nephrotoxicity of routine contrast agents (gadolinium chelates) at common doses. Image quality is good with high spatial, temporal, and contrast resolution. Another advantage is the versatility to perform different types of imaging that highlight specific anatomy or functions, making MRI equally suitable for angiography, flow quantification, wall depiction, or even brain imaging in the same setting. Complete carotid circulation including extracervical segments can be examined, and 3D acquisitions can be evaluated. Furthermore study has shown that there is a high correlation between sonographic CIMT measurements with mean wall thickness measured by MRI which is potential to have higher reproducibility (Underhill *et al.*, 2006). However patients need to be capable of lying flat for a longer period of time and follow simple instructions, as movement will result in image quality degradation. Severe claustrophobia, obesity, advanced kidney disease and incompatible metallic implants or electronic devices are also contraindicated in MRI. In addition, the availability of MRI is usually limited as compared to ultrasound, and the cost is much higher.

2.6 Sonographic B-Mode Examination of Carotid Intima-Media Thickness (CIMT)

Pignoli *et al* in 1986 has shown that CIMT sonographic measurement did not differ significantly from the intima-medial thickness measured on pathologic examination. By using a high frequency transducer, longitudinal B-scan of the common carotid artery is performed and the distance between two echogenic lines in the far wall of the artery is measured as CIMT (Pignoli *et al.*, 1986). As technology advanced, near wall and far wall of the carotid artery can be visualised by ultrasound (Figure 2.6), however B-mode evaluation of the near wall is less reliable than the far wall (Touboul, 2002). This can be explained by the ultrasound physic principal in which the anatomic location of an interface corresponds to the image interface only when the ultrasound beam comes from a less more dense tissue (Plavnik *et al.*, 2000). Thus the leading edge of the near wall has no anatomic relevance between the media-adventitia and the lumen interface. For young adult CIMT measurement, the reproducibility is greatest when combining values from both carotid arteries (Gonzalez *et al.*, 2008).

A meta-analysis of 8 observational studies which involved 37,197 subjects who were followed up for a mean of 5.5 years revealed that by increment of 0.1mm in CIMT, the future risk of stroke increases by 13-18% and the myocardial infarct risk increases by 10-15% (Lorenz *et al.*, 2007). Conditions associated with increased

CIMT are familial hypercholesterolemia, hypertension, obesity, diabetes, metabolic syndrome, HIV, Kawasaki disease (Urbina *et al.*, 2009).

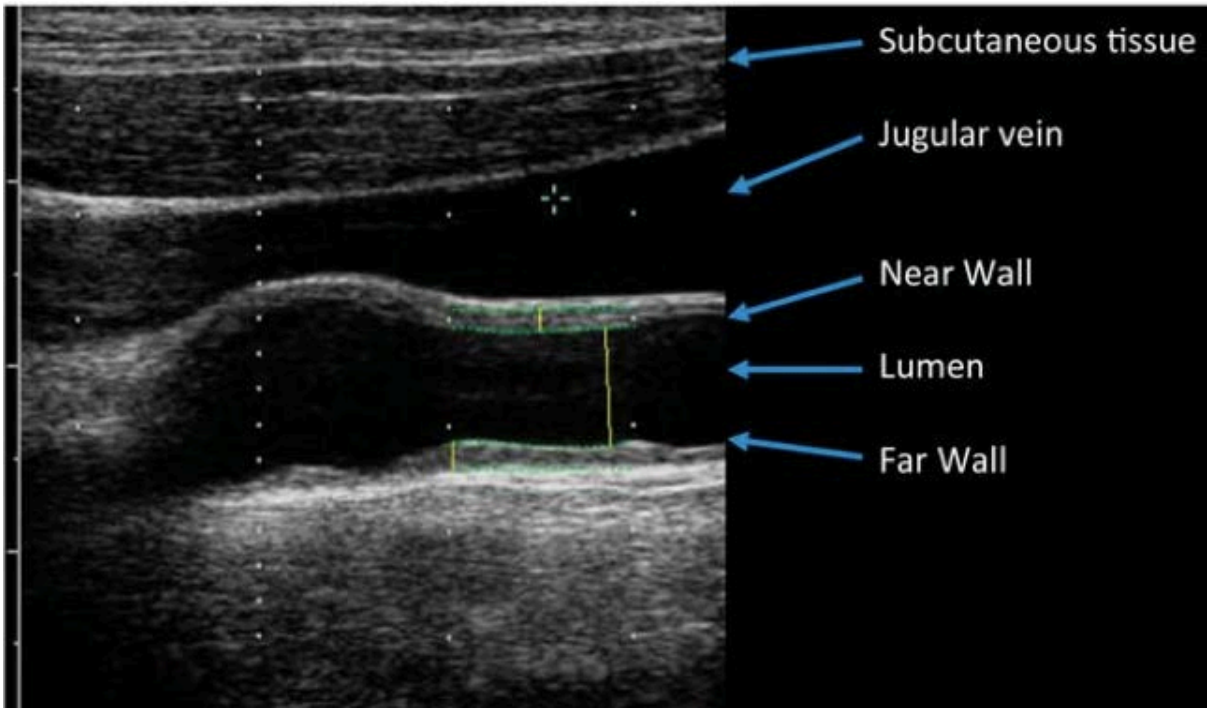


Figure 2.7: B-mode sonography of common carotid artery showing near wall and far wall. (Adapted from O'Leary and Bots, 2010)

3.0 OBJECTIVES

3.1 General Objective:

To measure carotid intima media thickness among thalassemia patient in HUSM

3.2 Specific Objectives:

1. To compare sonographic CIMT measurement between thalassemia patients in HUSM and non- thalassemic population.
2. To look for association between CIMT measurements of thalassemia patient in HUSM and patient's age, disease duration, number of blood transfusions and serum ferritin level.

3.3 Hypothesis

Null hypothesis:

There is no difference in sonographic CIMT value between thalassemia patients in HUSM and non- thalassemic population.

4.0 VALIDATION STUDY

4.1 INTRODUCTION

A validation study was performed prior to main research to validate the researcher's measurement of intima-media thickness and to determine its reproducibility. Both inter and intra-observer variability was tested in this study.

4.2 OBJECTIVES

The study was aimed to determine the inter-observer and intra-observer reproducibility of the IMT measurements performed by the researcher.

4.3 METHODOLOGY

The study was conducted in Department of Radiology, Hospital Universiti Sains Malaysia, Kubang Kerian using Siemen Acuson S2000 ultrasound machine with a 18 Mhz linear array transducer. The focus, depth and gain were standardized by employing the incorporated program within the ultrasound equipment.

Ten healthy volunteers were recruited from friends and staff from Department of Radiology, Hospital Universiti Sains Malaysia for the validation study. All volunteers were non-smoker with no history of hypertension, diabetes,

hypercholesterolemia, not on oral contraceptive pills and no history of neck trauma. The procedure was explained to all the participants and verbal consent was obtained. Two observers were involved in this study: the researcher, (observer A) and a consultant radiologist, (observer B).

Each subject underwent two examinations on the same day by observer A to determine the intraday reproducibility and other examinations by observer B to determine the inter-observer reproducibility of the intima-media thickness recordings. The observers were blinded to each other's findings. All the volunteers underwent another examination on a different day to determine the interday reproducibility of observer A recordings.

4.3.1 Technique

4.3.1.1 Subject preparation

Subject was rested for 10 minutes before proceeding with ultrasound examination

4.3.1.2 Subject positioning

i. Subject positioned on supine position.

ii. The ipsilateral shoulder is drop as far as possible and subject's head is rotated away from the side of being examined for maximum neck area exposure for the ultrasound examination.

iii. To facilitate vessel visualization, subject's head and neck position was adjusted accordingly during the ultrasound examination

4.3.1.3 Measurement

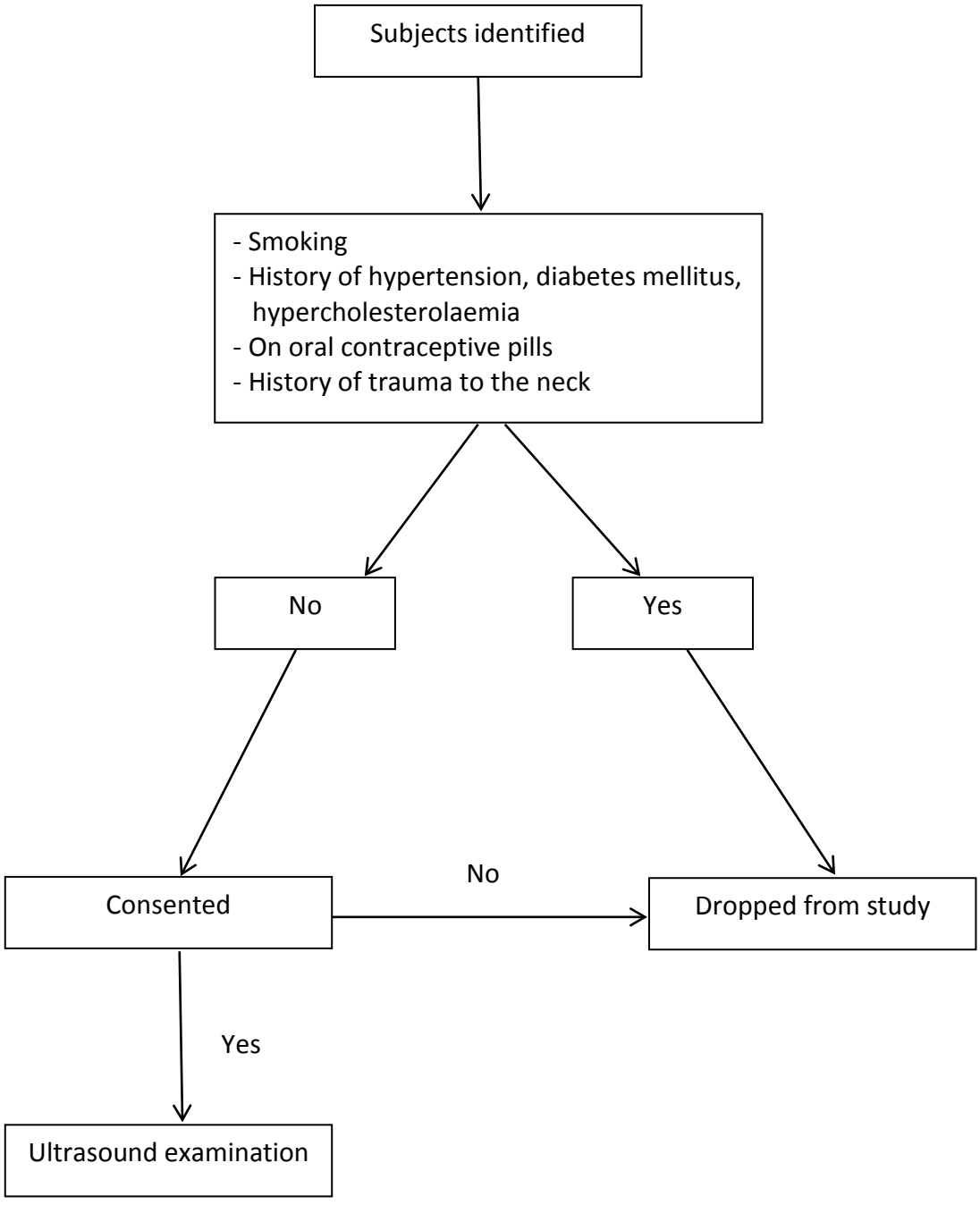
The measurement of intima-media thickness was made in longitudinal plane of common carotid artery at the point of 1.0cm from the carotid bulb. Carotid bulb is defined as the point where the far wall deviated away from parallel plane of distal common carotid artery.

Intima media thickness is defined as the distance between two echogenic lines located at the far wall. The first echogenic line represents the blood-intima interface while the second echogenic line represents media-adventitia junction. After freezing the image, the measurement was made with electronic calipers. The screen was unfreezed and the site of measurement was relocated before another measurement was made. Three measurement were made on each side and mean value was then calculated.

4.3.2 Statistical Method

All data were validated using IBM SPSS Statistic software (v 22.0) for Windows.

4.4 FLOW CHART



4.5 RESULT

For intra-observer reliability, reliability analysis was performed with Case 1 ICC (1): one-way random model, single measure. Three measurements were made for each subject on each side of the neck. For left neck, the intraclass correlation was of 0.67 (95% CI: 0.329, 0.892). As for the right neck, the intraclass correlation was of 0.62 (95% CI: 0.263, 0.873). Results are summarized in table 4.1.

Table 4.1: Intra-observer reliability with Case 1 ICC (1): one-way random model, single measure.

	Intraclass correlation (single measure)	**95% Confidence interval**
Left neck	0.67	0.329, 0.892
Right neck	0.62	0.263, 0.873

For inter-observer reliability, reliability analysis for the measurement made by researcher and consultant radiologist was performed with Case 3 ICC (A, 1): two-way mixed model, single measure. For left neck, the intraclass correlation obtained was of 0.946 (95% CI: 0.809, 0.986) and for the right neck, the intraclass

correlation obtained was of 0.964 (95% CI: 0.87, 0.991). Results are summarized in table 4.2.

Table 4.2: Inter-observer reliability with Case 3 ICC (A, 1): two-way mixed model, single measure.

	Intraclass correlation (single measure)	95% Confidence interval
Left neck	0.946	0.809, 0.986
Right neck	0.964	0.87, 0.991

4.6 DISCUSSION

For left neck, the measurement made by researcher is of good reliability with Case 1 ICC (1) of 0.67 (95% CI: 0.329, 0.892). As for the right neck, the measurement made by researcher is of good reliability with Case 1 ICC (1) of 0.62 (95% CI: 0.263, 0.873).

As for inter-observer reliability, reliability analysis for the measurement made by researcher and consultant radiologist was performed with Case 3 ICC (A,

1): two-way mixed model, single measure. For left neck, the intraclass correlation obtained was of 0.946 (95% CI: 0.809, 0.986) which suggests a very good agreement between researcher and consultant radiologist. As for the right neck, the intraclass correlation obtained was of 0.964 (95% CI: 0.87, 0.991) which suggests a very good agreement between researcher and consultant radiologist.

In conclusion, this study described that the measurement of the intima-media thickness of the common carotid artery performed by researcher was as accurate as the consultant radiologist. Hence the author was eligible to perform the examination on his own.

5.0 METHODOLOGY

5.1 Study Design

Cross sectional study using ultrasound measuring carotid intima media thickness among patient with beta thalassemia in HUSM and non-thalassemic populations.

5.2 Reference Population

Hospital Universiti Sains Malaysia (HUSM)

5.3 Source Population

All thalassemia patients who is being treated or follow-up in HUSM that fulfill all the criteria.

5.4 Study Period

19 months (January 2013 – August 2014)

5.5 Place of Study

Department of Radiology, PPSP, USMKK

5.6 Ethical Consideration

Ethical clearance and informed consent were taken for all patients who were eligible for the study. This study is fully explained to the subjects by the researcher and signed informed consent forms (Appendix 1) were kept for record.

5.7 Sampling Method

Convenient samplings in which all thalassemia cases from haematology ward or clinic are screened for the study. Subjects who are eligible will be given Questionnaire A before proceeding to ultrasound examination.

5.8 Inclusion and Exclusion Criteria

5.8.1 Inclusion Criteria

1. For thalassemia patient: all thalassemia patients who seek treatment in HUSM
2. For non-thalassemia population: all non- thalassemia individuals from general population.

5.8.2 Exclusion Criteria

1. Subject who has being diagnosed for hypercholesterolemia, hypertension, diabetes mellitus, metabolic or connective tissue disease.
2. Subject who is on anti- coagulant, oral contraceptive pills and bed ridden
3. Subject is a smoker.
4. Subject who has history of trauma to the carotid arteries.

5.9 Research Tools

1. Questionnaires

   A short questionnaire, Questionnaire A (Appendix 2) will be given to each eligible subject before proceeding to ultrasound examination.

2. US machine Siemens Acuson S2000 (Siemens, Erlangen, Germany)
3. Medical records

5.10 Sample Size Calculation

Objective 1

Using PS Power and Sample Size Calculations software (Version 3.0.1, January 2009), sample size were calculated based on 2 means.

$$n = \frac{2\sigma^2}{\Delta^2}(Z\alpha + Z\beta)^2$$

$\alpha = 0.05$

Power, $Z\beta = 0.8$

Difference of CIMT measurement between thalassemia and normal population, $\Delta = 0.1$ (G.Hahalis et al, 2008)

Standard deviation of CIMT among thalassemia patient, $\sigma = 0.07$ (G.Hahalis et al, 2008)

Sample size calculated = 32/ group

20% dropout = 38 samples/ group

Objective 2

Using Stata (Version 11.0) software, sample size for objective 2 is calculated based on regression analysis (age variable).

Alpha, α	= 0.0500 (two sided)
power	= 0.8000
alt sloop of age (β)	= 0.4000 (G.Hahalis et al, 2008)
Residual sd	= 7.0305
Standard deviation of age	= 7.0000 (G.Hahalis et al, 2008)
Correlation between CIMT and age	= 0.3700 (G.Hahalis et al, 2008)

Calculated sample size, n = 50/ group

20% dropout = 60 samples/ group

Total sample size: 60 samples/ group

5.11 Data Collection and Statistical Analysis

5.11.1 Objective 1

Scan technique

Examinations were performed with subjects in supine position after ten minutes rest period. Volunteers were asked to drop the ipsilateral shoulder as much as possible and to tilt and rotate their head away from the side of examination to maximize exposure of the region of

interest. Adjustment to the position of the subject head and neck was made during the examination to facilitate vessels visualization. Researcher sat on the right side of the patient (figure 5.1); with the ultrasound gel applied on the ultrasound probe, examination of the common carotid artery were commenced by putting the probe in the anterolateral position of the subject's neck. Both common carotid arteries were examined in their full visible length.

Measurement

In B-mode ultrasonography, several layers of echogenicity representing layers of vessel wall can be visualized. Because of inability to differentiate between the intima and media layer in ultrasound, the measurement of the combined thickness of intima and media is used, known as intima-media complex. All IMT measurements were made in longitudinal plane. The point with the maximum thickness on the far wall of common carotid artery along

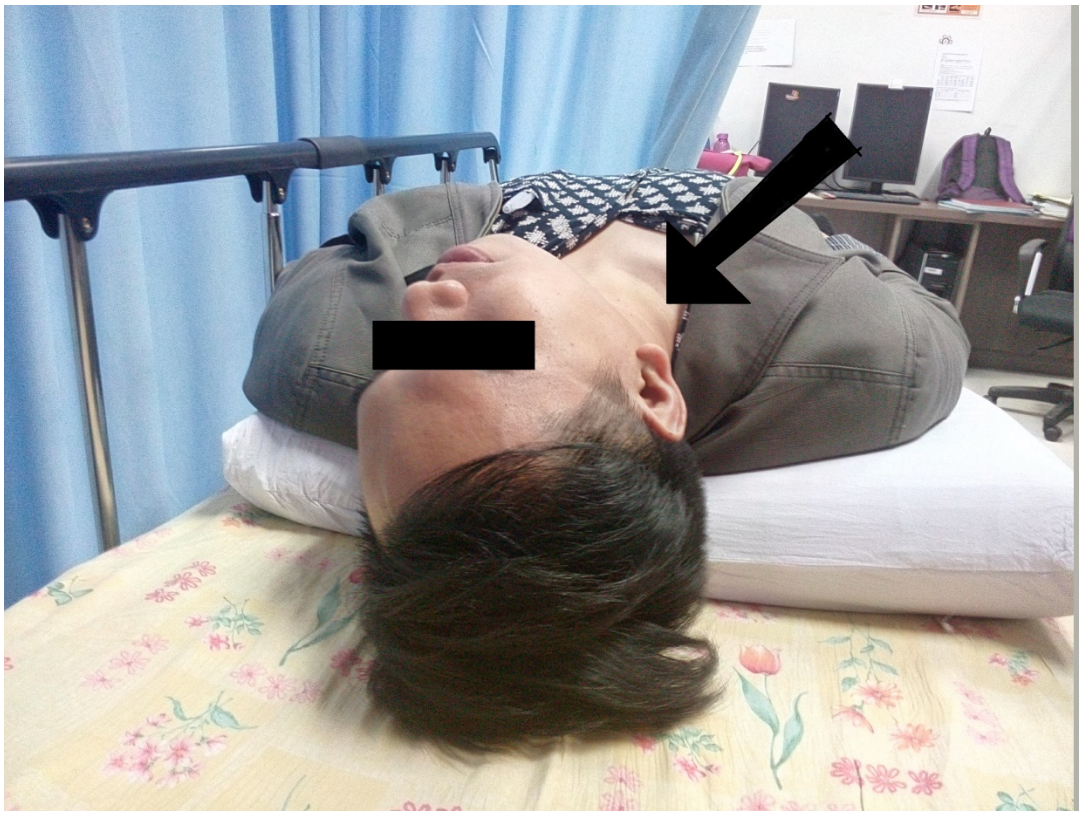


Figure 5.1: Patient positioning for the ultrasound examination. Arrow indicates ultrasound probe placement.

1cm section proximal to the carotid bulb is taken. After freezing the image, the measurement was made with electronic calipers. Magnification of the ultrasound image was used to improve accuracy of placement of the calipers. The first echo along the far wall is derived from the lumen/intima interface, while the second echogenic line represents the media/ adventitia interface (Sidhu and Desai, 1997). The combined intima- media is thus the hypoechoic region between the two echogenic lines (figure 5.2). Measurements were repeated three times on each side. On each occasion, image will be unfreeze and relocated to the position of the maximal intima-media thickness. A total of 6 readings for each subject will be taken. The mean values of the 6 readings are taken representing the mean IMT for the subject. All data were recorded on the data collection sheet (Appendix 3 and 4). Data were analyzed for mean and standard deviation for each group (thalassemia and general population). Then, data normality and homogenicity of variance were checked using histogram and Levene's test respectively before using independent T-test. All data analysis were done using IBM SPSS Statistic software version 22.0 for Windows package program. P value of less than 0.05 was considered as significant.

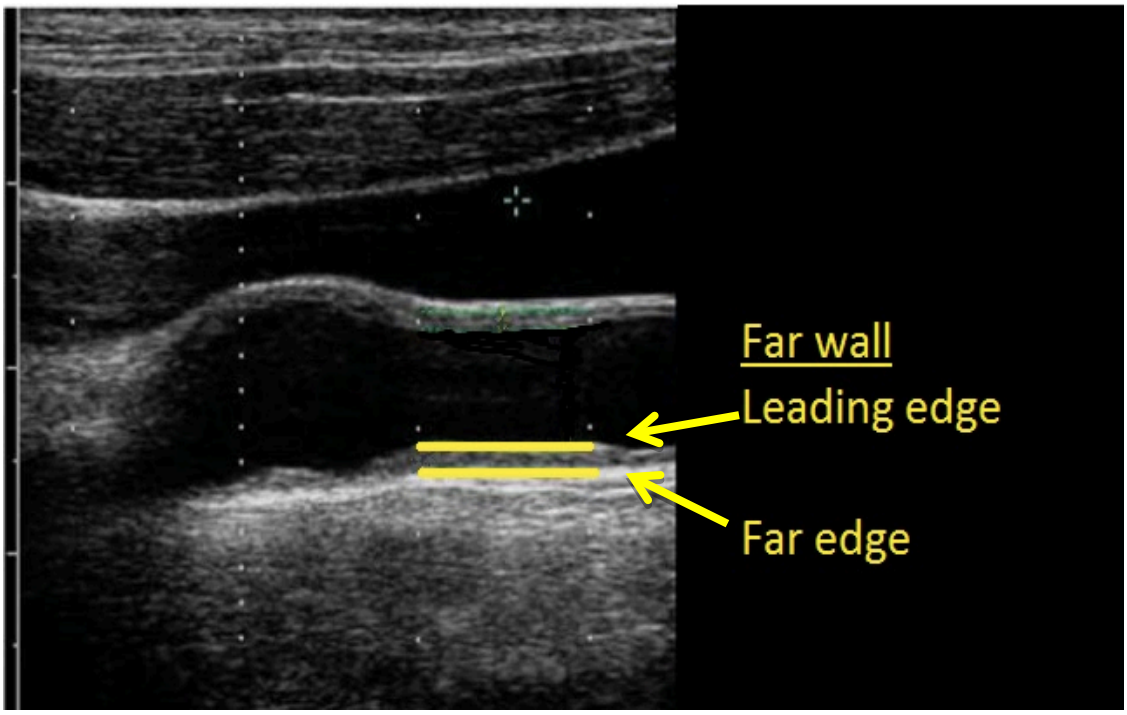
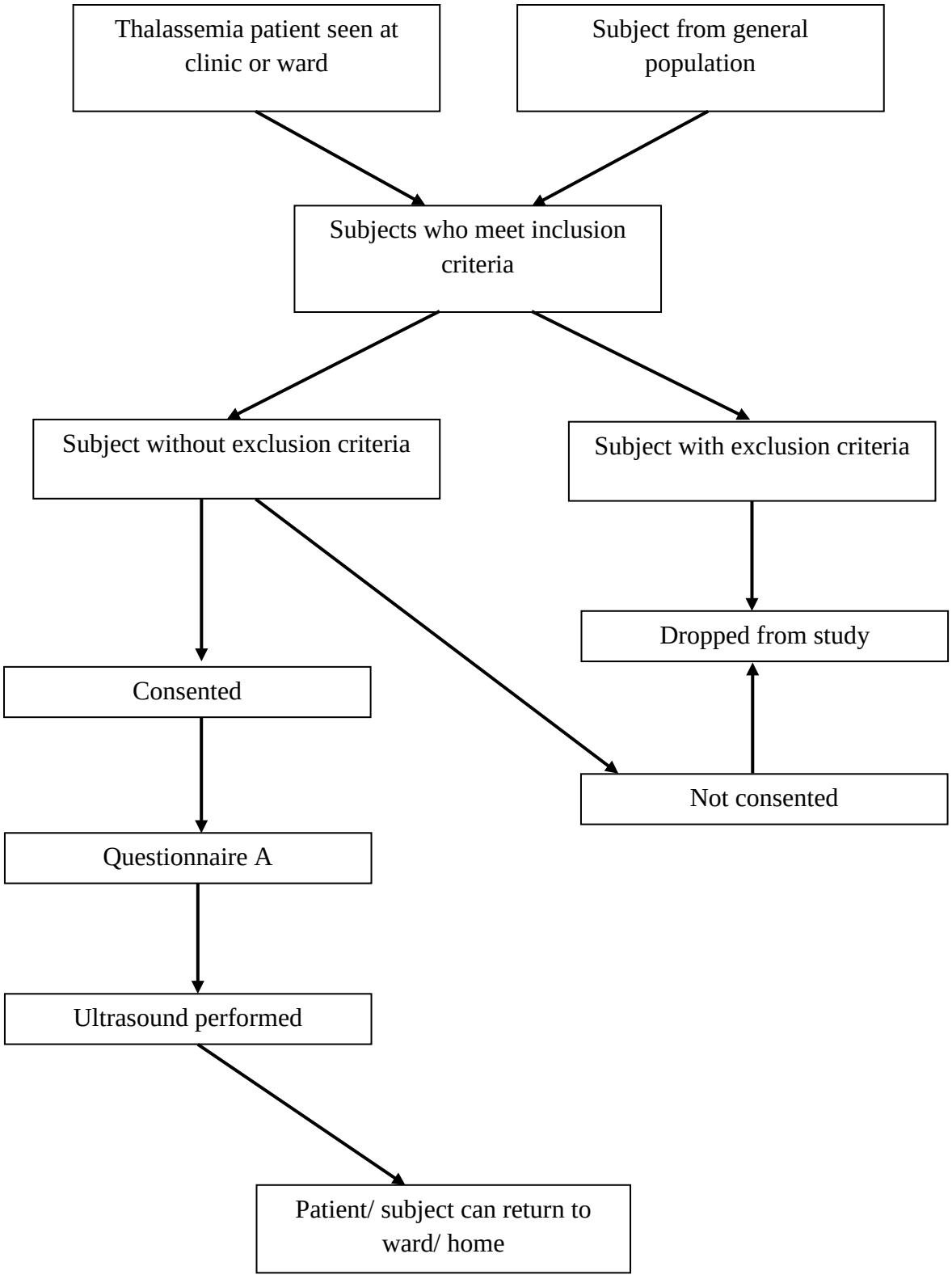


Figure 5.2: Carotid intima-media thickness measurement on the far wall, proximal to the carotid bulb. The distant between the 2 yellow lines is taken as CIMT measurement. (Adapted from O'Leary and Bots, 2010)

5.11.2 Objective 2

Thalassemia patient's age, disease duration, number of blood transfusion and serum ferritin level are collected from questionnaire A (Appendix 2) and patient's medical record folder which were recorded on the data collection sheet (Appendix 4). The data were analyzed statistically for any association with patient's CIMT measurement using multiple linear regression analysis. IBM SPSS Statistic software version 22.0 for Windows will be used.

5.12 Flow Chart



6.0 RESULTS

Data analysis was performed using IBM SPSS software version 22.0 for Windows. Prior conducting the desired test, descriptive analysis was performed to summarize the nature of the collected data. Overall, 80 samples were collected. There were equal samples representatives from non- thalassemia group as well as thalassemia group.

6.1 Demographic Data

In the non- thalassemia group, 52.5% of the subjects are female while the rest 47.5% are male (Figure 6.1); with Malay contributes up to 67.5% while the rest of the subject are Chinese (32.5%) (Figure 6.2). The mean age for the non- thalassemia group is 19.1 years (Figure 6.3).

For the thalassemia group, male subjects are slightly higher with 52.5% compared to female (47.5%) (Figure 6.4). Malay contributes up to 92.5% in the thalassemic group; followed by Chinese (5%) and Siamese (1%) (Figure 6.5). Mean age of the collected samples is 15.7 years old (Figure 6.6).

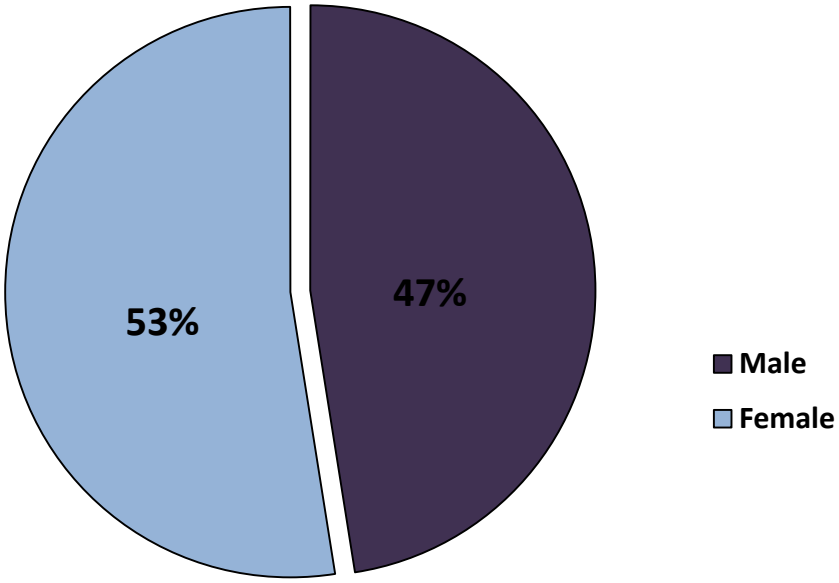


Figure 6.1: Gender distribution among non- thalassemia group

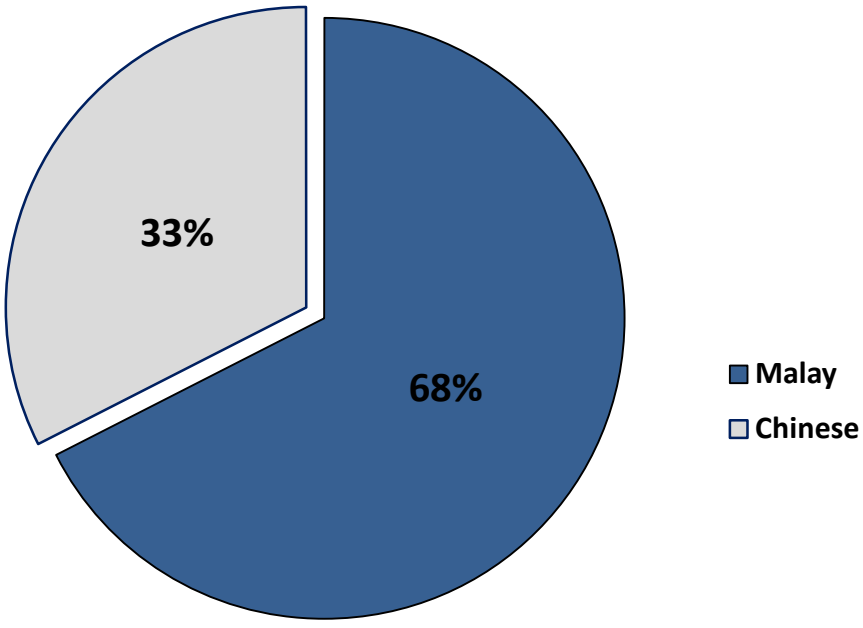


Figure 6.2: Race distribution among non-thalassemia group

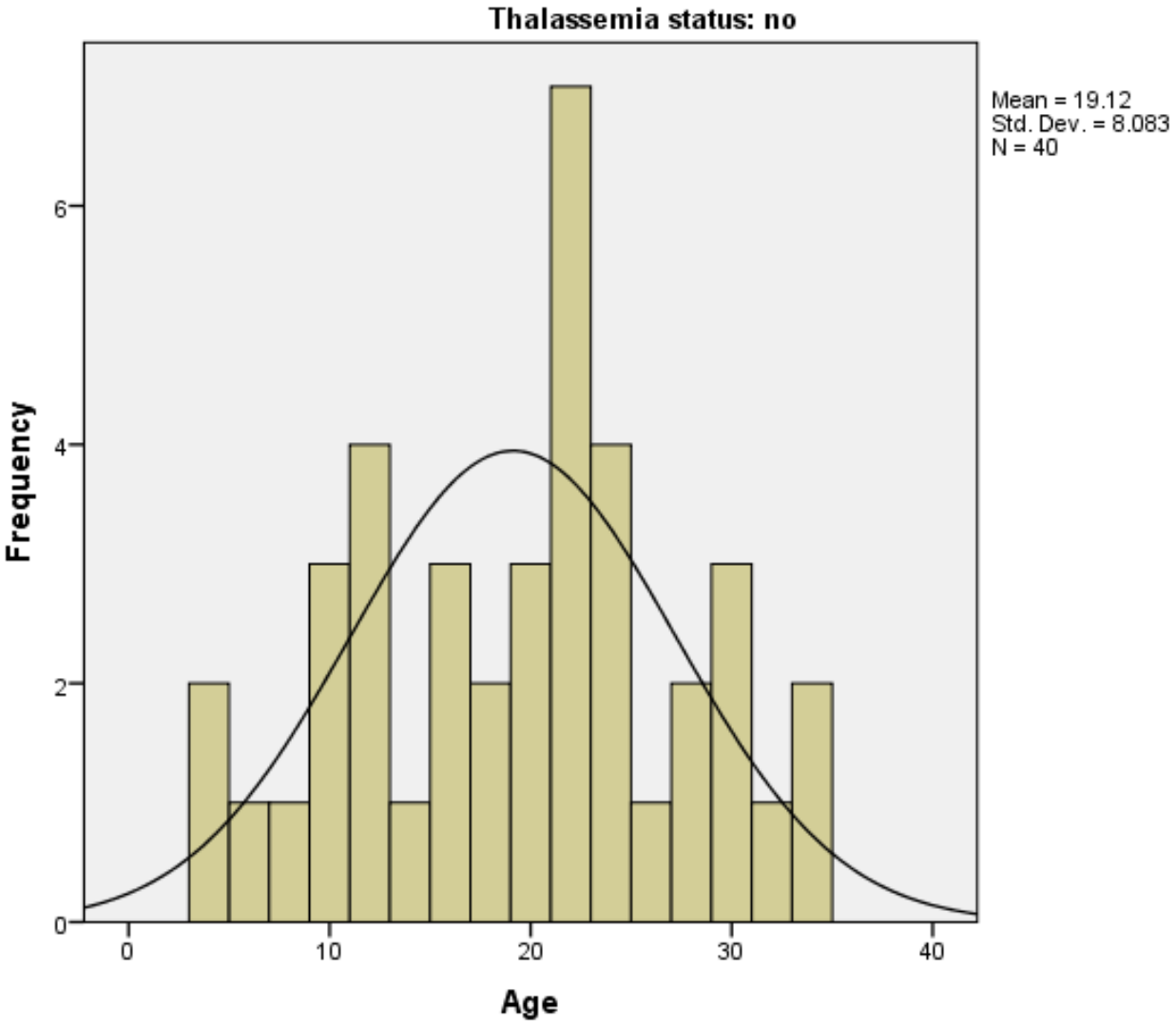


Figure 6.3: Mean age among non- thalassemia group.

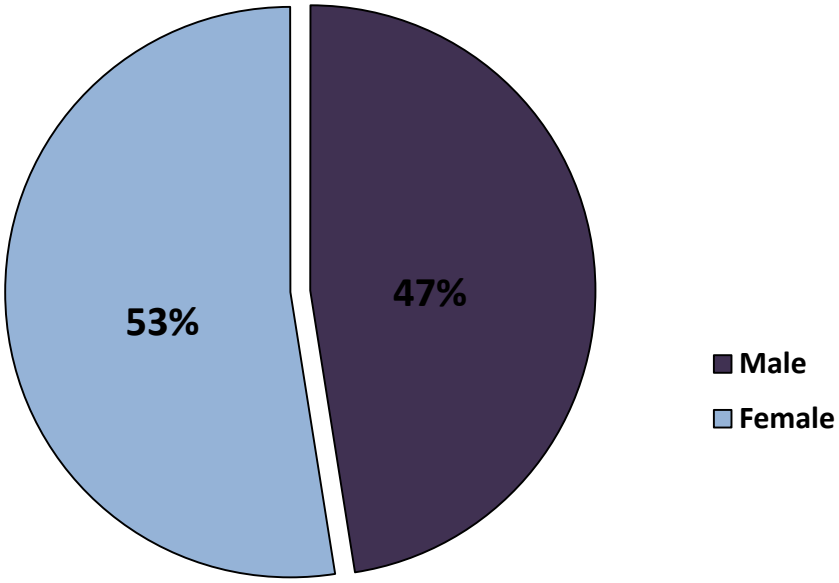


Figure 6.4: Gender distribution among thalassemia group

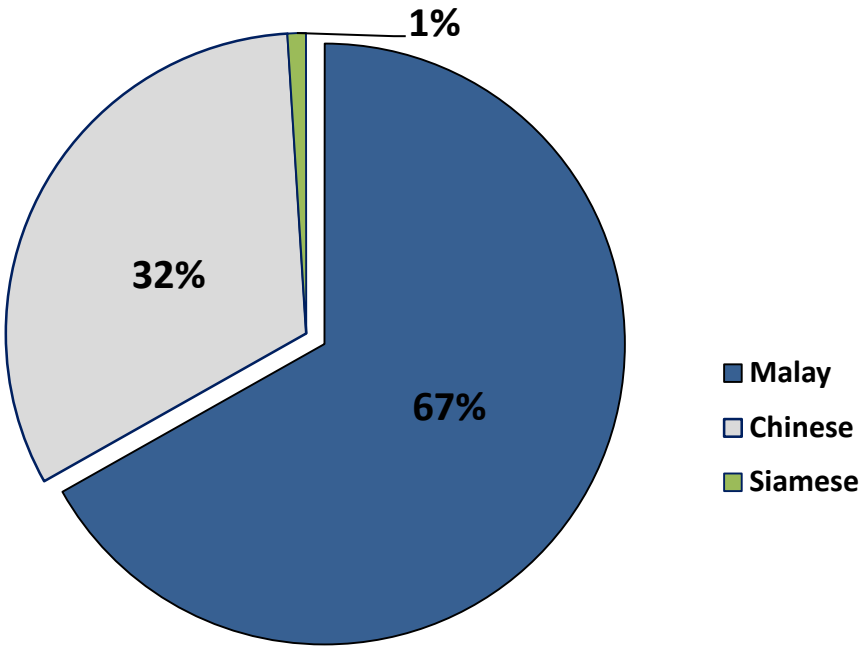


Figure 6.5: Race distribution among thalassemia group

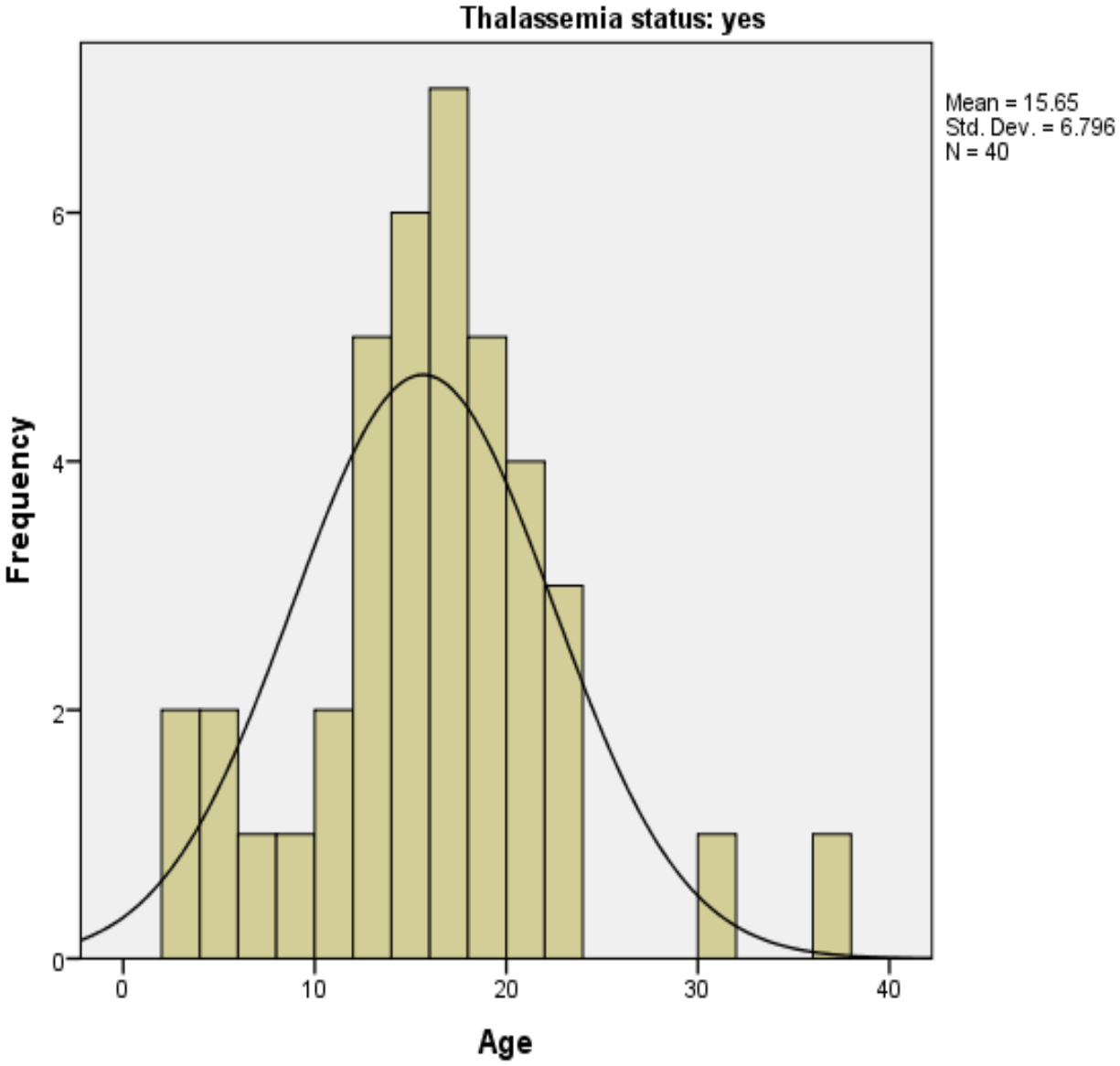


Figure 6.6: Mean age among thalassemia group.

6.2 Comparison CIMT measurement between thalassemia group with non-thalassemia group

From our study, we found that the mean value of the intima- media thickness of the common carotid artery in non- thalassemia group is 0.32mm. The mean value of the intima- media thickness of the common carotid artery in thalassemia group is slightly thicker than non- thalassemia group measuring 0.45 mm.

An independent t-test was done to compare sonographic CIMT measurement between thalassemia group and non- thalassemia group. Assumptions were checked before performing the test. Assumptions for random sample and independent observation were completed during study design stage. Then, normality of both groups is checked by using histogram and is found to be normally distributed as in Figure 6.7 and figure 6.8. Assumptions for homogeneity of the variance is checked by using Levene's Test and equal variance is assumed ($p = 0.093$).

Result for independent t-test is as shown in Table 6.1. With p value <0.001 and confidence intervals does not include 0, conclusion is made based on alternative hypothesis. We were 95% sure that the mean CIMT of those having Thalassemia (0.45) is significantly higher than those not having thalassemia (0.32) with the confidence interval lies between -0.17 and -0.09.

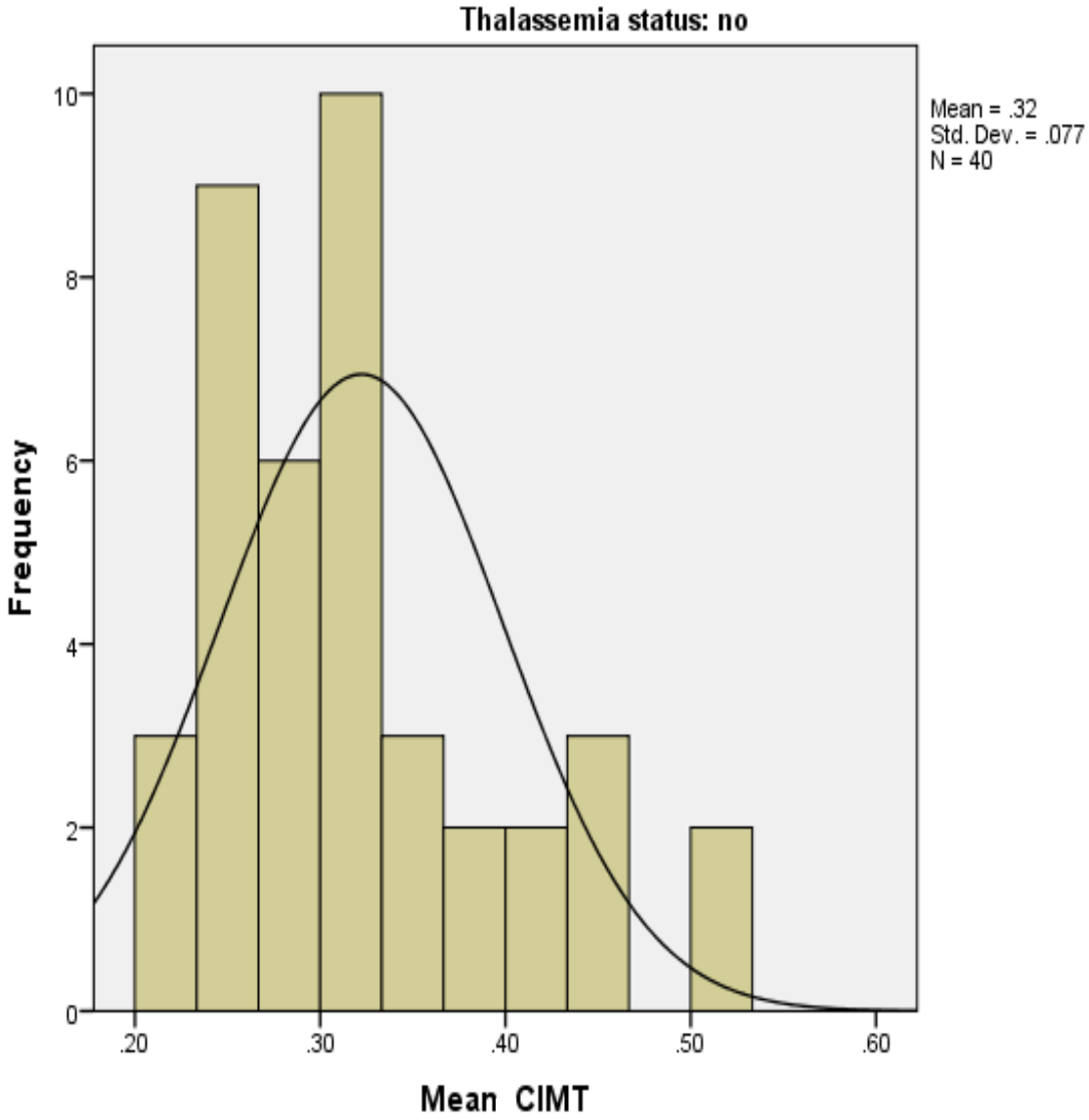


Figure 6.7: Mean CIMT among non- thalassemia group.

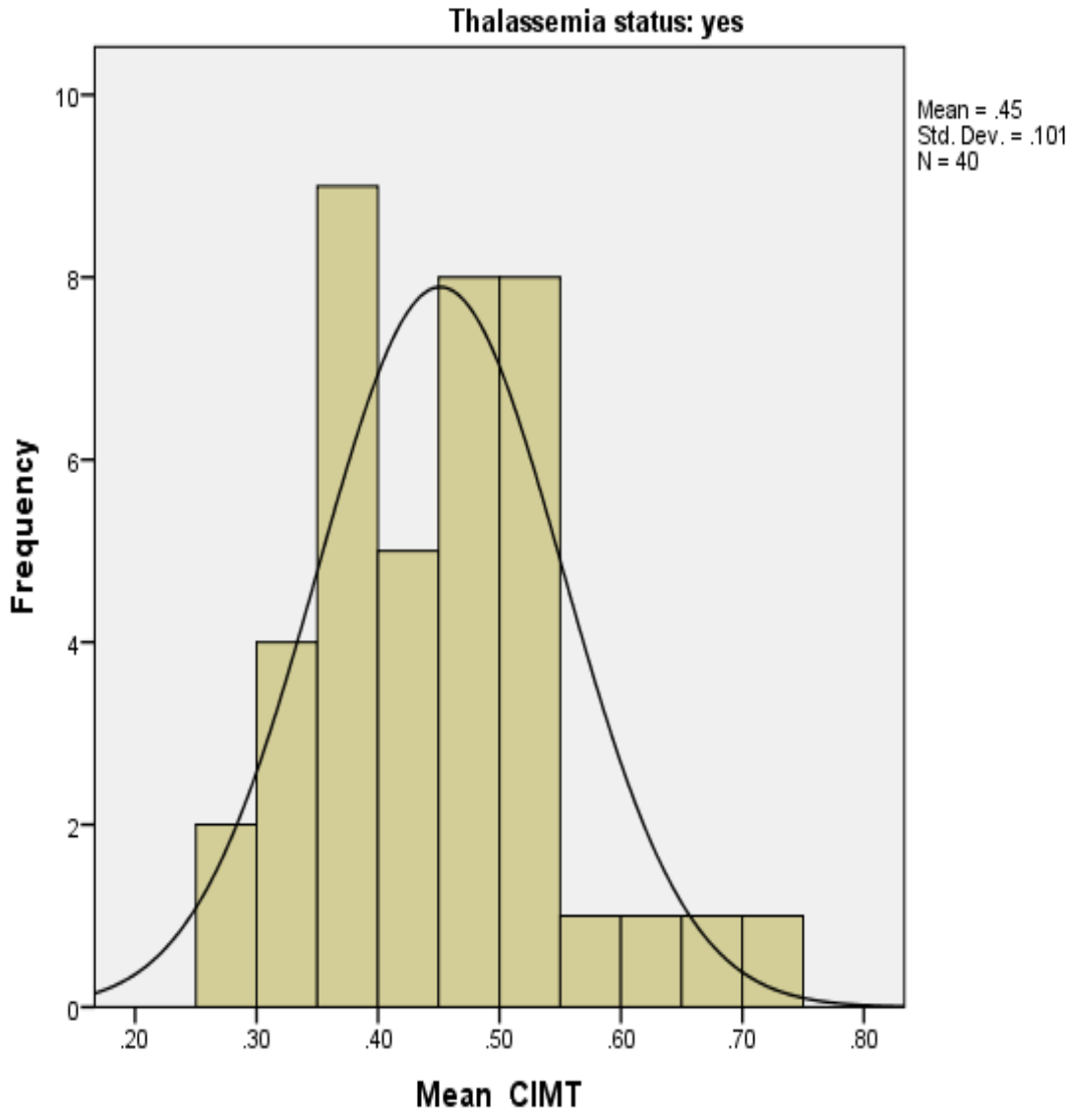


Figure 6.8: Mean CIMT among thalassemia group.

Table 6.1: Mean CIMT between thalassemia and non- thalassemia group (n=80)

Study subjects	**Variable mean CIMT (SD)**	**Mean difference (95 % CI)**	**t statistics**	***p* value**
Thalassemia group	0.45 (0.10)	-0.13 (-0.17, -0.09)	-6.43	<0.001
Non-thalassemia group	0.32 (0.08)			

6.3 Association between CIMT and patients' age, disease duration, numbers of blood transfusion and serum ferritin level.

From the data that have been collected, the mean value of disease duration of the thalassemia patient is 11.8 years with mean value of serum ferritin level is 4447.0ng/dL and mean value of number of blood transfusion is 131.8 times (Table 6.2).

To look for any association between mean CIMT measurements of thalassemia patient with patient's age, disease duration, number of blood transfusions and serum ferritin level, a multiple linear regression test (MLR) was performed. Scatter of plot of each variable against the outcome was plotted (figure 6.9, 6.10, 6.11 and 6.12) and separate simple linear regression (SLR) was performed prior performing the MLR.

Table 6.2: Thalassemia patient mean disease duration, serum ferritin level and numbers of blood transfusion

Variable	Mean (SD)
Disease duration (year)	11.8 (5.9)
Serum ferritin level (ng/dL)	4447.0 (2642.6)
Numbers of blood transfusion (times)	131.8 (11.8)

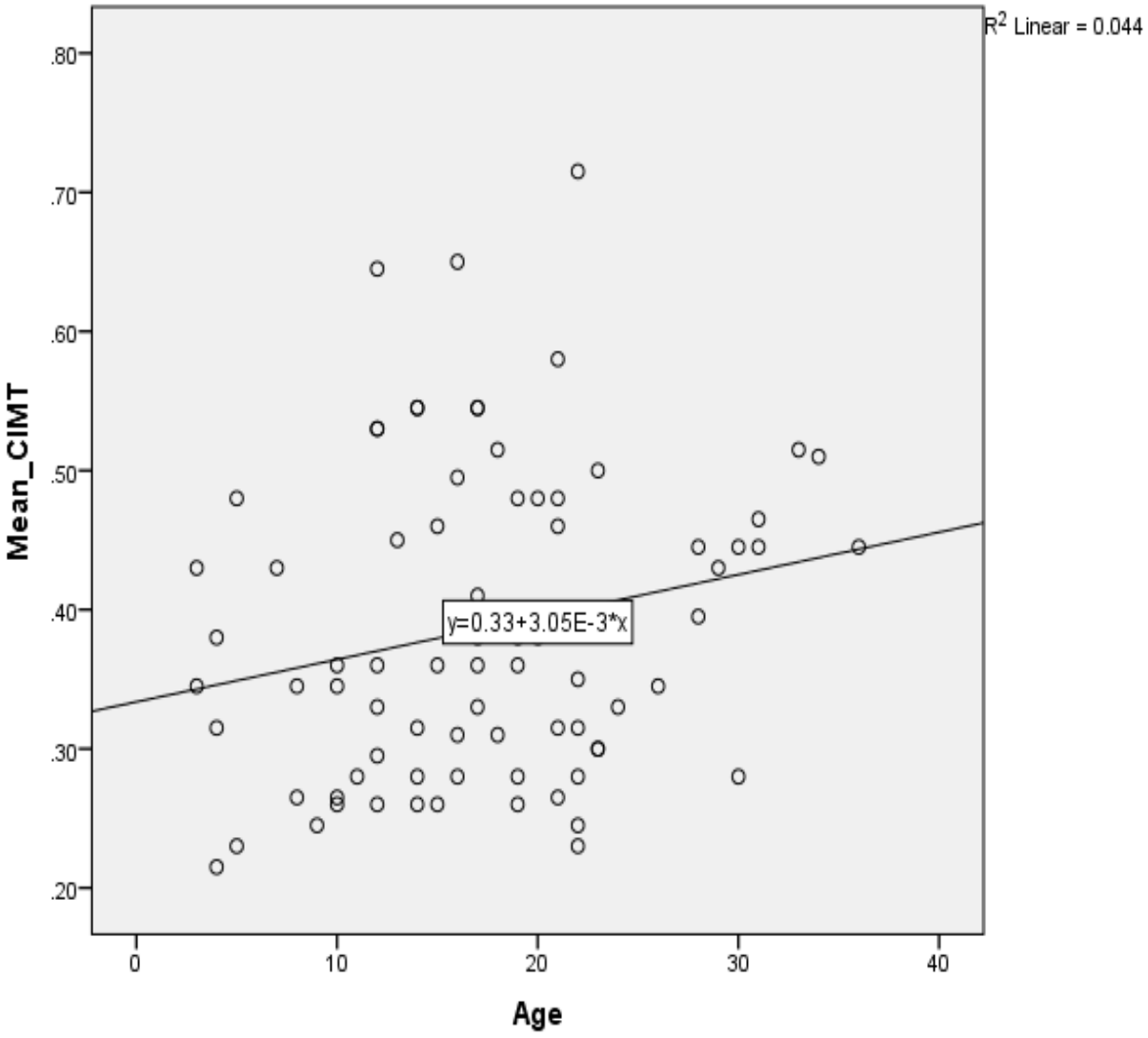


Figure 6.9: Age against mean CIMT

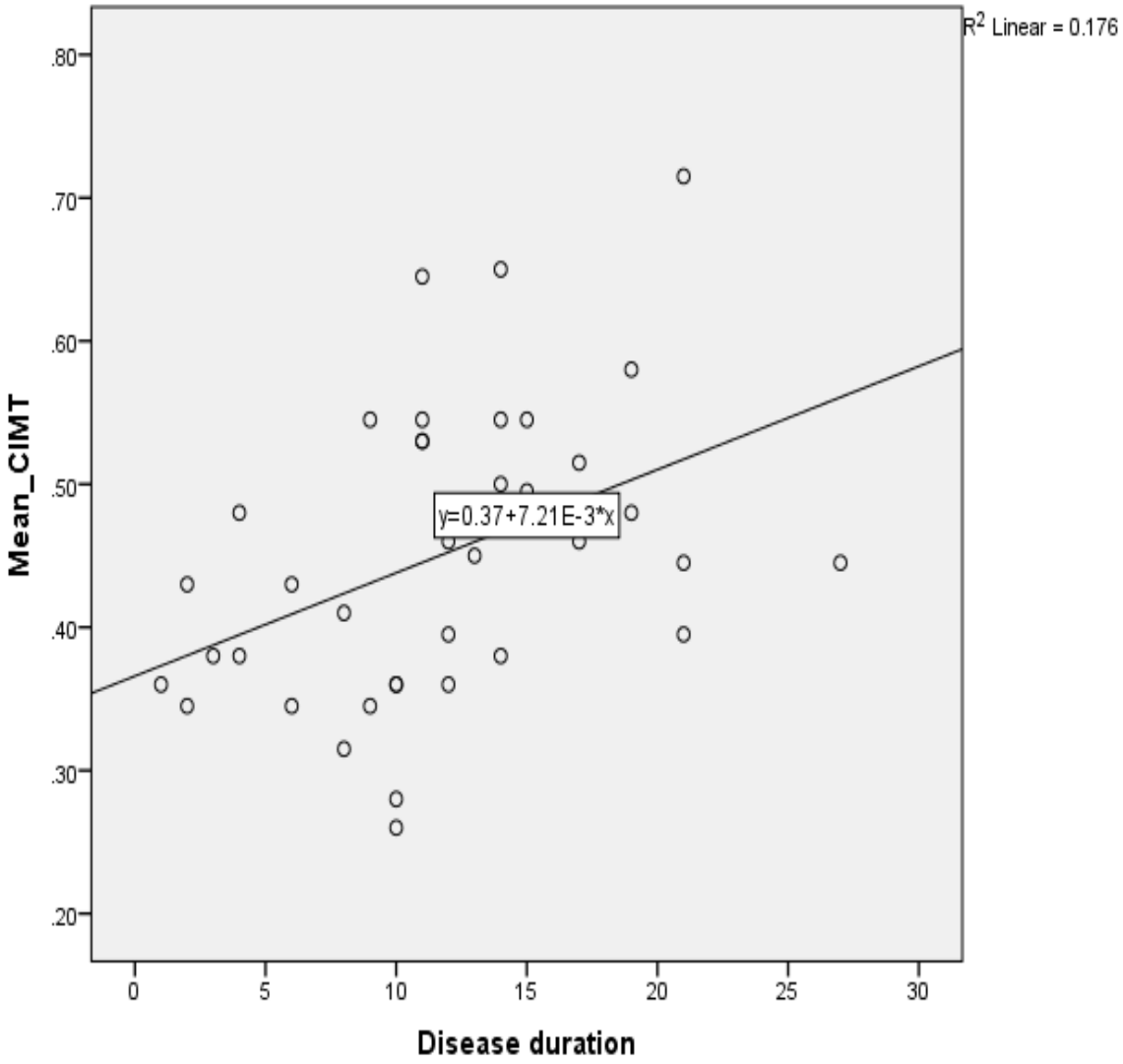


Figure 6.10: Disease duration against mean CIMT

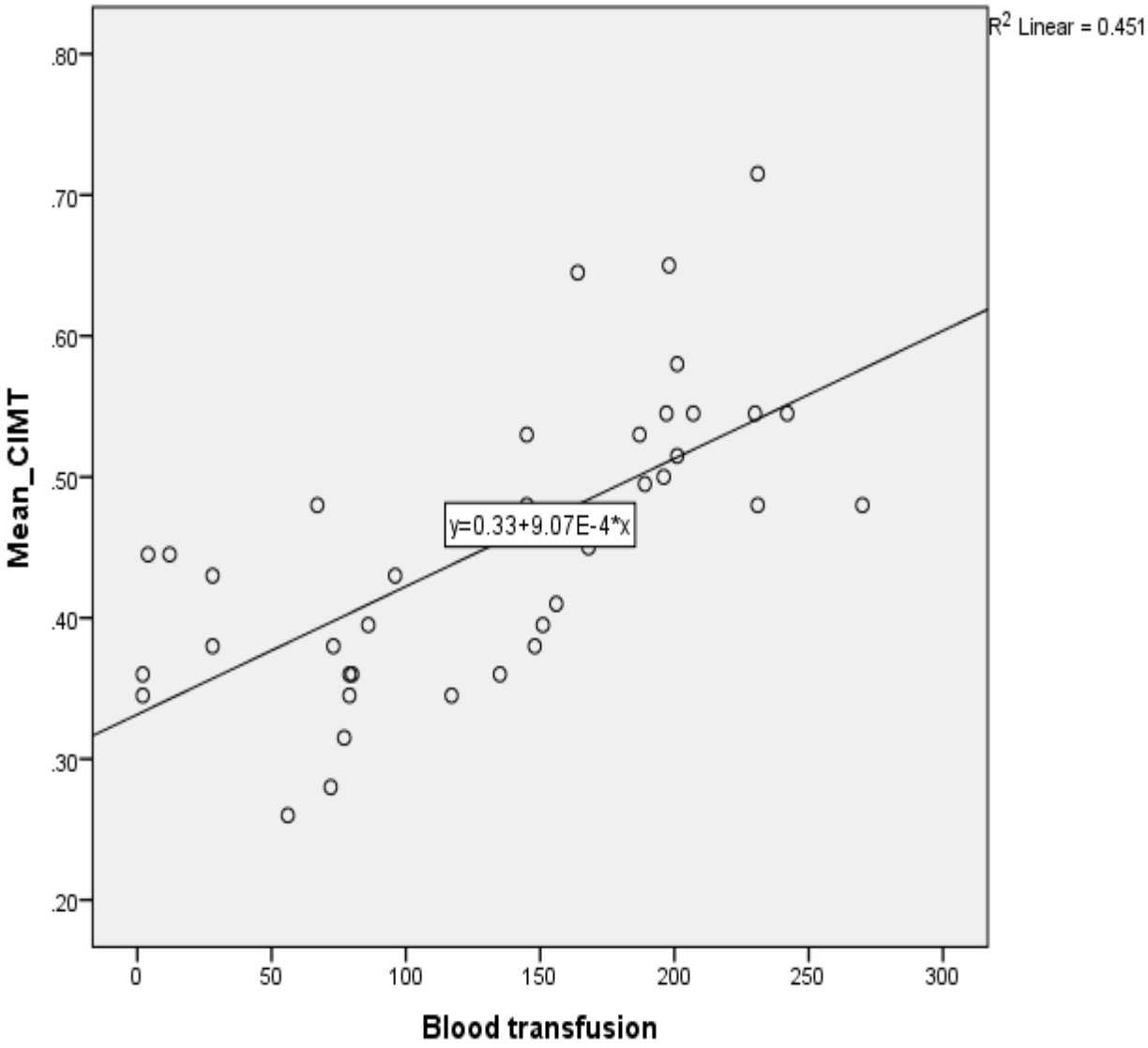


Figure 6.11: Number of blood transfusion against mean CIMT

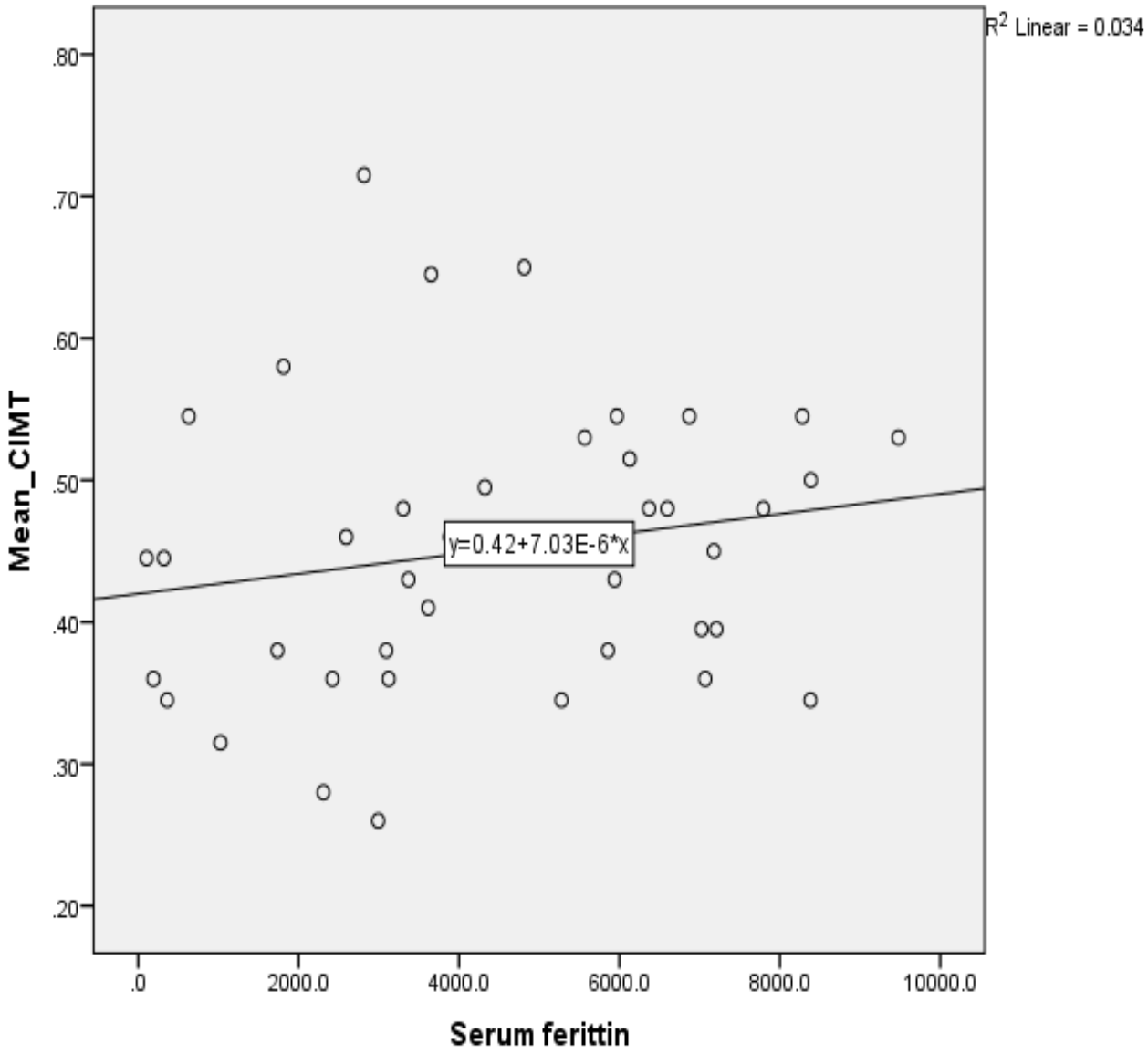


Figure 6.12: Serum ferritin level against mean CIMT

From the scatter plot, all four variables showed positive relationship with the mean CIMT. Further analysis using simple linear regression (SLR) was done. Results for the separate SLR are shown in table 6.3.

Table 6.3: Association between age, disease duration, numbers of blood transfusion and serum ferritin and mean CIMT among thalassemia patient (n=40) using simple linear regression test.

Variable	***b** (95% CI)**	**t statistics**	***p* value**	**r^2**
Age	0.003 (0, 0.06)	1.9	0.06	0.21
Disease duration	0.007 (0.002, 0.012)	2.85	0.007	0.42
Numbers of blood transfusion	0.001 (0.001, 0.001)	5.59	<0.001	0.67
Serum ferritin	$7x10^{-6}$ (0.00, 0.00)	1.153	0.256	0.18

* crude regression coefficient

For the multiple linear regression tests, variables selections into the model were performed using stepwise, forward and backward methods. Only variable number of blood transfusion is found to be significant in the model (Table 6.4).

Table 6.4: Factor associated with mean CIMT among thalassemia patient (n=40) using multiple linear regression test.

Variable	*b** (95% CI)	t statistics	*p* value	r^2
Numbers of blood transfusion	0.001 (0.001, 0.001)	5.59	<0.001	0.67

* crude regression coefficient

This MLR test requires few assumptions to be fulfilled. Scatterplot of unstandardized residual versus unstandardized predicted value were plotted as in Figure 6.13 to check if its linearity and equal variance are met. Independent observation was settled during study design stage. Normality of the residual was also checked and is normally distributed as in Figure 6.14. Assumptions of relationship between residual and variable number of blood transfusion was also checked by plotting the scatter plot and is met as in Figure 6.15. Since all assumptions are met, the final model for second objective is achieved. Number of blood transfusion is the only variable found to have association with mean CIMT.

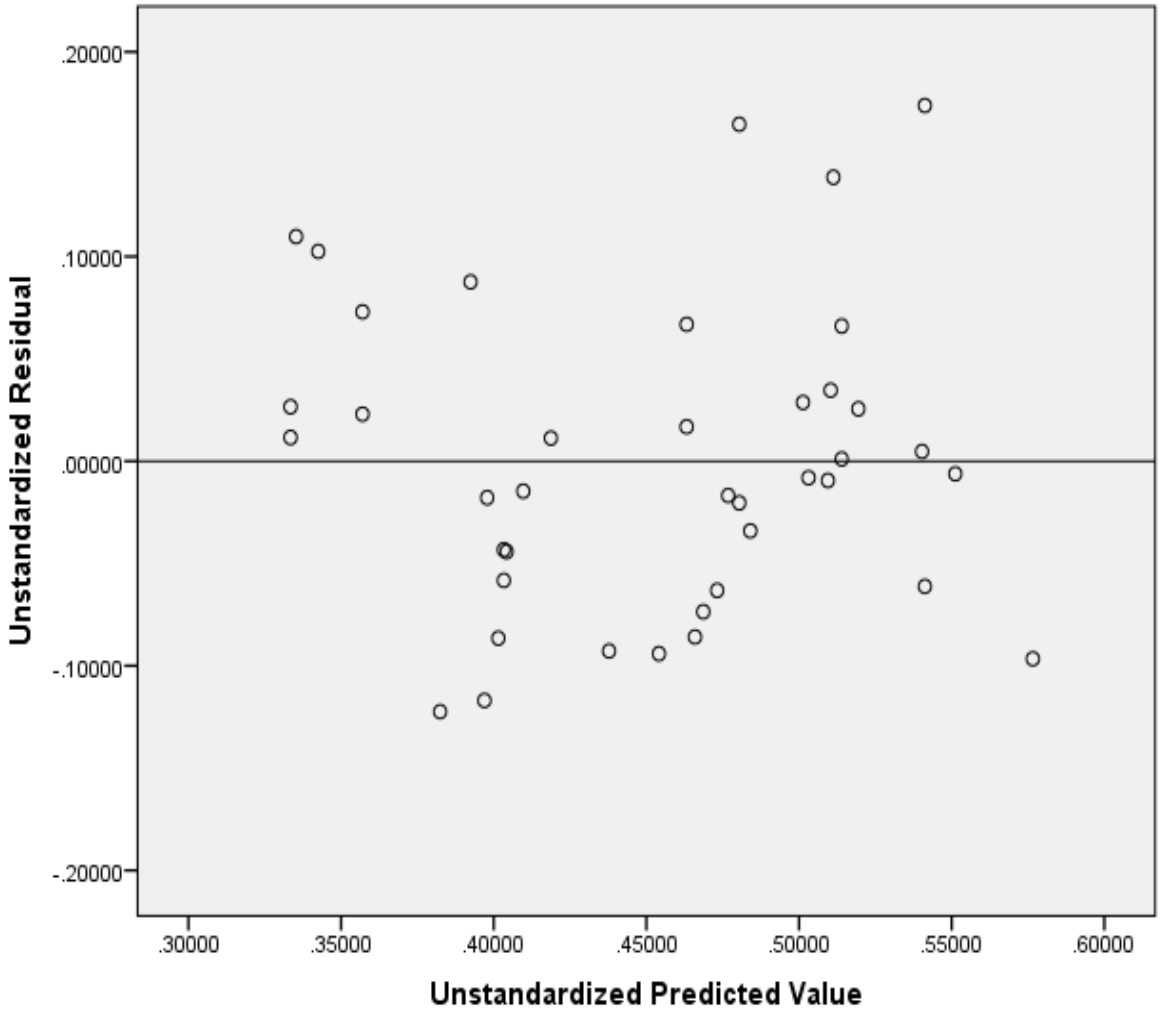


Figure 6.13 Assumption: Unstandardized residual against unstandardized predicted value

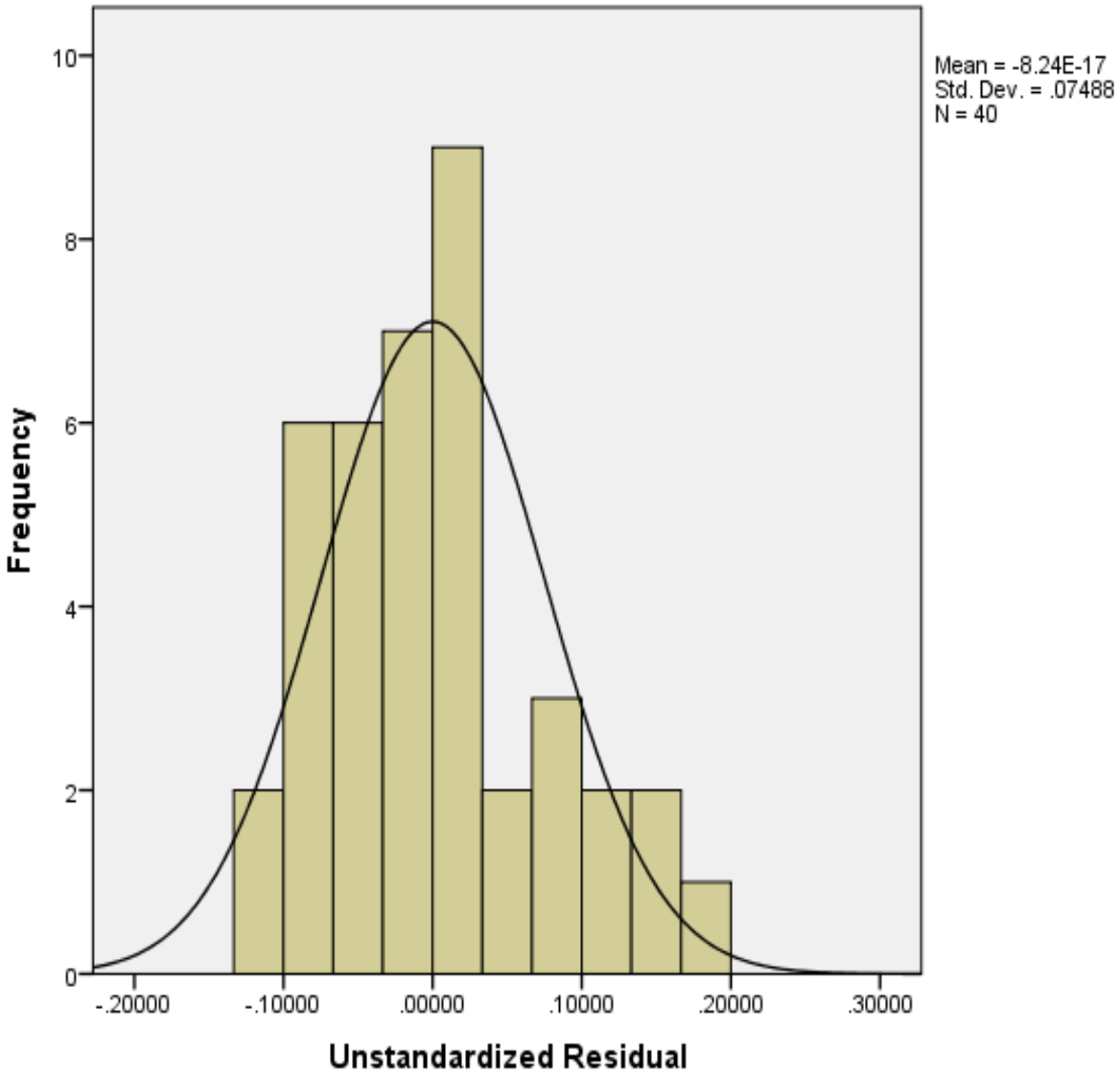


Figure 6.14 Assumption: Normality of residual

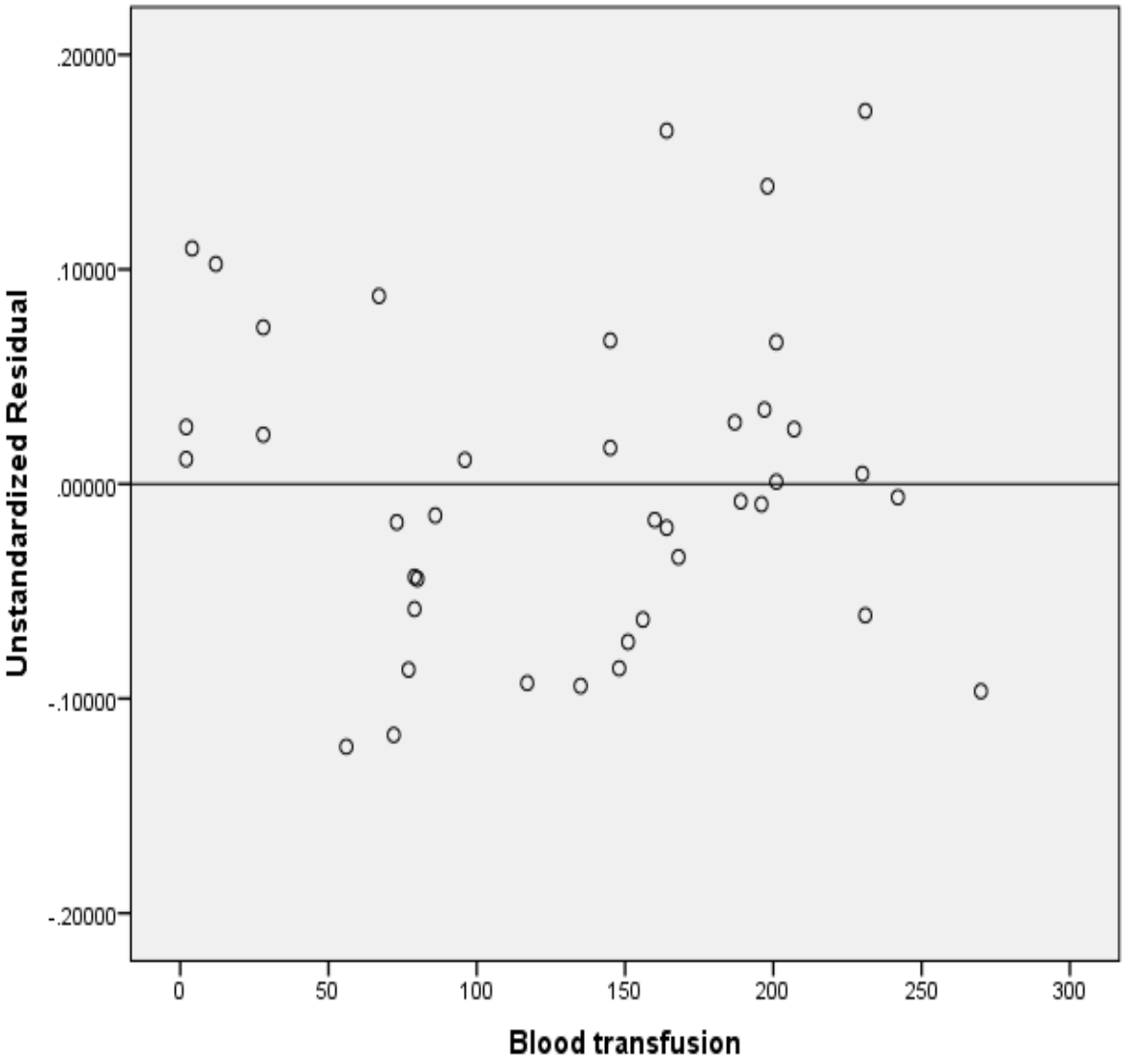


Figure 6.15 Assumption: Unstandardized residual against numbers of blood transfusion

7.0 DISCUSSION

7.1 Overview

Thalassemia patient lifespan nowadays has been increased significantly compared to previously due to the advancement of the medical treatment and better healthcare system. As a result, more transfusion-related complications had been seen among the thalassemic patients which are associated with increased in iron overload. One of the rising trends seen in thalassemia patient is the thromboembolism complication. The incidence of stroke in beta thalassemia major patients ranges from 2% to 20% according to studies (Borgna Pignatti *et al.*, 1998). Another study shows that up to 29% of beta thalassemia intermedia developed either deep vein thrombosis, pulmonary embolism or portal vein thrombosis during a 10-year follow up (Cappellini *et al.*, 2000).

There are few methods that had been advocate for the assessment of iron overload in thalassemia patient. These include serum ferritin, liver biopsy and liver MRI. Serum ferritin although is an easy and cheap investigation had been found to be inaccurate in assessment for iron overload. Liver biopsy is an invasive method and depends on technicality for correct assessment. MRI T2* imaging is non-invasive technique with high reproducibility for iron overload assessment. A study done in Iran showed there was a correlation between liver iron overload severity based on liver MR T2* imaging and sonographic CIMT measurement (Akhlaghpoor *et al.*, 2010).

Sonographic CIMT measurement is a recognised surrogate marker for future cardiovascular events (Bots *et al.*, 1997). It is increasingly being used to assess cardiovascular risk as atherosclerotic disease in the carotid artery has been associated with both coronary and cerebrovascular disease (Craven *et al.*, 1990).

There have been few studies investigating CIMT measurement in thalassemia patient which show a significant increased than normal population and there are few factors that have been associated with the CIMT increment. Sonographic CIMT measurement is also recommended to be done in beta thalassemia patients as a non-invasive early diagnostic tool (Ismail and El-Sherif, 2010; Dogan and Citak, 2011) and for vascular risk stratification (Tantawy *et al.*, 2009).

There is no similar study done on sonographic CIMT measurement on thalassemia patient in in Southeast Asia, particularly Malaysia. This study would be helpful because of the different type of thalassemia in Malaysia which mainly comprised of HbE β-thalassemia, which is a structural β-globin Hb variant with a β+ phenotype (George, 2013). HbE β-thalassemia shows highest frequencies in Asia such as India, Bangladesh, Thailand, Laos, Cambodia (Olivieri *et al.*, 2011) and also in Malaysians' Malay population (George, 2013).

7.2 Demographic Characteristic

Our study has managed to include a total of 80 subjects, which consist of 40 thalassemia and 40 non- thalassemia subjects. Our thalassemia subjects is slightly more than previous studies which had been done in Italy (Cusmà Piccione *et al.*, 2013), Turkey (Dogan and Citak, 2011; Gursel *et al.*, 2012), Egypt (Tantawy *et al.*, 2009; Ismail and El-Sherif, 2010), Greece (Hahalis *et al.*, 2008) and Hong Kong (Cheung *et al.*, 2006) which ranges from 20 to 36 subjects. Our study managed to get higher subject samples than previous study possible due to we include all type of thalassemia and did not restrict our subjects only to beta thalassemia major like other studies. The sex distribution is almost equal between male and female with the mean age of 15.65± 6.8 years.

The mean age for other studies are almost similar to our study except for Cusmà Piccione *et al.*, 2013 and Hahalis *et al.*, 2008 which involves older subjects (mean age of 35± 8years and 27± 7years respectively). Other studies recruit slightly younger subjects such as Dogan and Citak, 2011(median age of 8 years) and Ismail and El-Sherif, 2010 (mean age of 7.2± 3.4years). Our normal subjects (n=40) also almost equally distributed between male and female, with mean age of 19.13± 8.1 years.

Based on the race, our thalassemia study sample comprises 92.5% of Malays (n=37), 5% Chinese (n=2) and 2.5% Siamese (n=1). The majority of Malay subjects is likely due to the Malay race predominance in Malaysia which is followed by Chinese and Indian (Elizabeth and Ann, 2011).

7.3 Comparisan between thalassemia CIMT measurements and non-thalassemia group.

According to our study, the mean thalassemia CIMT measurement was 0.45± 0.1mm as compared to non- thalassemia group which measured 0.32± 0.08mm. From the data analysis, we found that thalassemic CIMT measurement was significantly higher than the non- thalassemia group. This was in concordance with other studies that had been done previously by Cheung *et al*., 2006 (0.45± 0.04mm vs 0.39± 0.02mm), Hahalis *et al.*, 2006 (0.51± 0.07mm vs 0.46± 0.07mm), Tantawy *et al.*, 2009 (0.73mm vs 0.63mm), Ismail and El-Sherif, 2010 (0.46± 0.08mm vs 0.35± 0.03mm), Dogan and Citak, 2011 (0.87mm vs 0.74mm) and Gursel *et al.*, 2012 (0.56± 0.06mm vs 0.48± 0.05mm).

The increased in the CIMT could be attributed by the hypercoagulability state in thalassemia. Among the causes of the hypercoagulability that have been identified are abnormal platelets, abnormal red blood cells, decreased in coagulation inhibitors, iron overload, hyperviscosity, endothelial damage, genetic mutations, cardiac and liver dysfunction and hormonal deficiencies (Eldor and Rachmilewitz, 2002; Panigrahi and Agarwal, 2007; Taher *et al.*, 2008; Cappellini *et al.*, 2010).

7.4 Correlation between thalassemia CIMT measurements and patient's number of blood transfusions.

Previous study had observed that there was a correlation between thalassemia patient's sonographic CIMT measurements with patients' number of blood transfusions. Our study also tried to show an association between the CIMT measurements with patient's number of blood transfusions.

From the single linear regression test, we had found that there was a significant linear relationship between thalassemia mean CIMT measurement and the number of blood transfusion ($p < 0.001$). It was observed that increasing in number of blood transfusion by 100 times will increase the mean CIMT by 1mm. On further analysis using multiple linear regression tests, we concluded that the numbers of blood transfusion was the only independent factor that significantly associated with patient's CIMT measurement. Our finding was in harmony with study done by Dogan and Citak, 2011. The study which was done in Turkey compared 33 beta thalassemia major patients which include 22 boys and 11 girls with median age of 8 years old with 30 healthy children. Another study done in Egypt by Ismail and El- Sherif in 2010 which involved 15 beta thalassemia major patients also showed there was a significant difference in CIMT measurement in relation to frequency of blood transfusion.

This was likely related to increase in iron overload as the frequency of blood transfusion is increased (McLeod *et al.*, 2009). The finding by Cusmà Piccione et al., 2013 which found there is no significant difference in CIMT measurement between general populations with non-iron overload thalassemia patient further supports our finding. Iron stores plays a strong role in atherogenesis (Kiechl *et al.*, 1997) and iron overload is thought to be one of the factors attributed to increased hypercoagulability in thalassemia patient.

Iron chelating agents had been introduced to blood transfusion depended beta thalassemia patient to reduce the iron overload. Desferoxamine is the first iron chelating agent that was introduced which was given through subcutaneous or intravenous route and studies had shown that it has a role to reduce the cardiovascular complication. However the discomfort it produced during administration and the exuberant treatment cost had an effect to its compliancy among thalassemia patient (Delea *et al.*, 2007; Dahlui *et al.*, 2009; Viprakasit *et al.*, 2009). As a result, new oral chelating agents had been introduced to increase the compliancy.

The exact mechanism of iron promoting atherogenesis is still unclear (De Valk and Marx, 1999). According to the 'iron hypothesis', iron is said to be proatherogenetic when it is in catalytically active form (Vinchi *et al.*, 2014). It participates in the formation of reactive oxygen species and induces lipid-

peroxidation, triggering endothelial activation, smooth muscle cell proliferation and macrophage activation.

7.5 Correlation between thalassemia CIMT measurements and patient's serum ferritin level.

From our study, the mean serum ferritin level of the thalassemia patients' measures 4470± 2642.6ng/ml. The measurement is slightly higher than previous study with the highest being recorded is 2860ng/ml (Dogan and Citak, 2011), followed by 2342.7ng/ml (Tantawy *et al.*, 2009), 2235ng/ml (Gursel *et al.*, 2012), and 1527ng/ml (Cheung *et al.*, 2006).

From our regression analysis, we did not find any association between patients' CIMT with their serum ferritin levels. Our finding corresponds with few studies (Cheung *et al.*, 2006; Hahalis *et al.*, 2008; Gursel *et al.*, 2012). However, there were few studies done in Egypt and Turkey showing an association between patient CIMT with serum ferritin levels (Tantawy *et al.*, 2009; Ismail and El-Sherif, 2010; Dogan and Citak, 2011).

The discrepancies in the result may lie in the limitations in serum ferritin levels in reflecting patient's true iron load status. Serum ferritin level represents only 1% of the total iron pool and as an acute-phase protein it is not specific (Argyropoulou and Astrakas, 2007). Serum ferritin level is affected by many factors such as chronic inflammation, infection process, liver disease and blood loss (Rossi *et*

al., 2000; Argyropoulou and Astrakas, 2007; Eghbali *et al.*, 2014). Our study did not exclude patient with hepatitis which is not rare in patients with thalassemia. Studies done in Pakistan (Din *et al.*, 2014), Egypt (Mansour *et al.*, 2011), Iran (Mirmomen *et al.*, 2006) and India (Manisha *et al.*, 2014) showed that about 49%, 40%, 19% and 18% of the thalassemia subjects have been inflicted with hepatitis C respectively.

Our study did not exclude patient with liver enzyme derangement. Study done by Cheng et al., 2006 and Hahalis et al., 2008 which show similar result as our study also did not exclude patient with impaired liver function test in their study. However studies done in Egypt and Turkey which exclude patient with hepatitis showed there were correlation between patient CIMT measurement and serum ferritin level (Tantawy *et al.*, 2009; Dogan and Citak, 2011). We believed that because of this, it may have an effect to the serum ferritin levels in our subjects thus affecting our study findings.

7.6 Correlation between thalassemia CIMT measurement with patient's age and disease duration.

Previous study has also found that there is an association between patients CIMT 'measurement with patients' age. A study in Greece which involved 35 beta thalassemia major patients with median age of 27± 7 years old and 35 normal control subjects showed that there was a positive correlation between CIMT and patients' age (Hahalis *et al.*, 2008). Tantawy *et al*. in 2009 also showed a positive correlation between CIMT and thalassemia patient's age. The study was performed on 30 beta thalassemia major patients with median age of 18.4± 6.2 years. Simliar finding was also noted in study done by Ismail and El-Sherif, 2010. Apart from that, study by Dogan and Chitak in 2012 had shown that there is a positive correlation between beta thalassemia patients' CIMT with disease duration.

From the univariate analysis in our study, we found that there is a significant linear relationship between thalassemic mean CIMT and disease duration ($p = 0.007$). It is observed that increasing in 50 years of disease duration will increase the value of CIMT reading by 0.35mm. However on further analyzing with multivariate test, we did not find any significant correlation between patients' CIMT with the disease duration. We also did not find any association between patients age with patient's CIMT measurement on the univariate analysis.

The reason behind the different finding in our study may be due to the type of thalassemia patients that were used as subjects. Other study only included thalassemia major in their study which has similar disease severity and needs regular blood transfusion. This is slightly differs from our study as we do not limit our subject to only thalassemia major. All thalassemia patients who were receiving treatment or follow-up in our hospital were included in the study. This would likely cause thalassemia minor patients as well as thalassemia intermedia patients to be included in our study. As a result, each patient in our study has different disease severity thus making their blood transfusion frequency varies. We believe that because of this, the iron overload burden will also varies between patients and not correlate with the age and disease duration of each patient, hence the incongruent result with other studies.

Apart from that, the commonest form of beta thalassemia in South-East Asia is Hb-E beta thalassemia (George, 2013). A study among 80 blood donors in Hospital Universiti Sains Malaysia also showed Hb-E beta thalassemia is the commonest form of thalassemia (Rosline *et al.*, 2006). The difference in the type of thalassemia from other studies might contribute to the result irregularities in our study, though this still need to be further clarified as we did not classified our subject according to their phenotype.

8.0 SUMMARY AND CONCLUSION

Evaluation of the carotid artery intima-media thickness with high resolution ultrasound is easy, although operator dependent, and is extremely useful to predict the risk of the cardiovascular and cerebrovascular disease. Sonographic CIMT measurement which is also a recognised surrogate marker for future cardiovascular events is highly reproducible technique for quantifying atherosclerotic burden.

From our study, we have found that there is significant increased CIMT measurement in thalassemia patient, indicating there is a higher risk of cardiovascular and cerebrovascular disease event compared to non- thalassemia group.

Our study also conclude that the number of blood transfusions is significantly associated with patient's CIMT measurement as being rationale by increased in the iron overload.

9.0 LIMITATION AND RECOMMENDATIONS

9.1 Limitations

The author has acknowledged several significant limitations of this, which include:

1. This study is operator dependent; however this single operator dependent has been overcome with validation study which was conducted prior to the validation study. Furthermore, the focus, depth and gain for all the ultrasound examination were standardized by employing the incorporated program within the ultrasound equipment.

2. The subjects who are being recruited in this study has mixed thalassemia clinical phenotypes, this could have an effect to the data collected compared to other studies which only specified to one clinical phenotype, thus will likely affect the end result.

3. The non-thalassemia group that had been recruited for this study are assumed to be normal based on the initial questionnaire assessment. No further clinical or blood parameters were done for confirmation. However, this can be negligible since hypertension, hyperlipidemia and diabetes mellitus is rare in younger age group.

4. Hypercoagulability in thalassemia is multifactorial. Other known confounding factors such as anti-thrombogenic agent (protein C, protein S), lipids and splenectomised status has not been included in this study.

5. The sample size collected in this study does is inadequate for the 2nd objective. This would likely affect the end result of this study.

9.2 Recommendation

The author suggests that the study to be done in bigger thalassemic population with proper grouping of the thalassemia subjects based on their phenotype for more accurate evaluation. The author also recommends that other co-founding factors for thalassemia hypercoagulability (protein C, protein S, lipids and splenectomised status) to be included if similar study were to be done in the future.

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APPENDICESS

Appendix 1:

STUDY INFORMATION

Study title : A study of carotid intima media thickness among thalassemia patients in HUSM

Name of researcher: Dr Ahmad Hadif Zaidin B Samsudin (MMC Registration Number: 43414)

Supervisor : Dr Juhara Haron (MMC Registration Number: 34234)

Co-Supervisor : Prof Madya Dr Ariffin Nasir (MMC Registration Number: 31850)

: Dr Rosnah Bahar (MMC Registration Number: 22368)

Introduction

You/ your child are invited to participate in a voluntary research study involving ultrasound examination of your neck to measure your carotid artery wall thickness. The wall thickness measurement has been used as risk stratification for thrombolic event (clotting of blood within the vessel). Before agreeing to participate in this research study, it is important that you read and understand this form. If you agree to participate, you will receive a copy of this form to keep for your records.

The purpose of this study

The aim of the study is to measure and compare sonographic carotid artery intima media thickness (CIMT) between thalassemias patient with the general population. An increased in carotid artery intima media thickness has been associated with increased risk of thromboembolic event. If you/ your child are a thalassemia patient, any association of CIMT with you/your child's age, disease duration, number of blood transfusion and serum ferritin level will be calculated. This would allow better thalassemias patient management in the future.

Entry qualification

The doctor in charge of this study or a research staff has discussed the requirements for participation in this study with you. It is important that you are truthful with the doctors and staff about your health history.

Requirements for this study are:
For thalassemia patients : You are seeking treatment/ follow up in HUSM
For general population : None

You cannot participate in this study if:

1. You have history of hypertension, diabetes, hypercholesterolemia or connective tissue or metabolic diseases.
2. You are a smoker.
3. You are taking anti- coagulant medication or oral contraceptive pills.
4. You are bed ridden or have history of trauma to the carotid arteries.

Study Procedures

If you agree to participate yourself/ your child for the study, a short questionnaire will be given. If you qualify to participate, an ultrasound examination of both sides of your/ your child's neck will be done. If you/ your child are a thalassemia patient, another short questionnaire will be given after the ultrasound examination asking more information on your/your child's disease duration and numbers of blood transfusion. Serum ferritin level will be review later from the medical record.

Ultrasound examination steps:

You/ your child will be asked to lie still on the bed with the head in slightly hyperextended position. An ultrasound examination of both sides of the neck will be performed. The whole examination may take 10-20 minutes in duration. You/ your child will be allowed to return home after that.

Risk:

As ultrasound wave is a non-ionising wave, therefore there is no risk of radiation and is it safe. If any new information found during this study may change the agreement and to continue to participate, you will be notified as soon as possible.

Reporting health expereinces.

If you have any injury, bad effect, or any other unusual health experience after the ultrasound examination, make sure that you immediately tell the nurse or Dr. Ahmad Hadif Zaidin B Samsudin [MMC Registration No:43414] at 09-7673468 or 013-9814800. You can call at anytime, day or night, to report such health experiences.

Participation in this study:

Your/ your child's participation in this study is voluntary. You can reject or terminated your/ your child's participation in this study at any time without penalty or loss of any benefit that would have been earned by you.

Possible Benefits

The study procedures will be provided at no cost to you. You may receive information about your health from any physical examination and laboratory tests to be done in this study. We hope that the outcome and information regarding this research will beneficial to future patients.

Questions:

If you have any questions about this study or your right, please contact;

Dr Ahmad Hadif Zaidin B Samsudin (MMC: 43414),
Department of Radiology, HUSM
School of Medical Sciences, USM Health Campus
Tel: 013-9814800
Email : hadif@kk.usm.my/ sam_hadif@yahoo.com

If you have any question regarding the approval ethical, please contact:

Mr. Mohd Bazlan Hafidz Mukrim
Secretary of Human Research Ethics Committee USM
Centre for Research Initiatives, Clinical & Health Sciences
USM Health Campus
Tel. No. : 09-767 2354 / 09-767 2362
Email : bazlan@usm.my/Jepem@kk.usm.my

Confidentiality:

Your/ your child medical information will kept confidential by doctor and staffs and will not be revealed unless is required by law. Your/ your child original medical records may be reviewed by ethical and regulatory authorities for the purpose of verifying the procedures or clinical research data. The medical information may be store in a computer or processed. By signing this consent form, your authorised the records, information storage and transfer of data as describe above.

Signature:

To be entered into the study, you or a legal representative must sign and data the signature page

Patient Consent Form

Title of study:	**A study of carotid intima media thickness among thalassemia patients in HUSM**
Name of researcher	: Dr Ahmad Hadif Zaidin B Samsudin (MMC Reg Number : 43414)
Supervisor	: Dr Juhara B Harun (MMC Reg Number : 34234)
Co-Supervisor	: Prof Madya Dr Ariffin Nasir (MMC Registration Number : 31850)
	: Dr Rosnah Bahar (MMC Registration Number: 22368)

To participate, you or legal representative must sign this page. By signing this page, I certify the following:

- I have read all the information in the Patient Information and Consent Form, including any information regarding the risk in this study and I have had enough time to think about it.
- All my questions were answered to my satisfaction.
- I voluntarily agree to participate in this research study, to comply with the study procedures and provide necessary information to doctor, nurses and other staff members as requested.
- I can terminate my participation in the study at any time.
- I have received a copy of the Patient Information and Consent Form to keep for myself.

Patient's Name	Patient number & initial
Patient Identification Card No (new)	Patient Identification Card No(old)
Patient's / legal representative's name/ IC and signature	Date(dd/MM/yy)
Researcher's name & signature	Date(dd/MM/yy)
Witness's name & signature	Date(dd/MM/yy)

Note: i) All subject/patients who are involved in this study will not be covered by insurance.

Appendix 2:

MAKLUMAT KAJIAN

Tajuk kajian: **Kajian tentang ketebalan 'carotid intima media' di kalangan pesakit thalassemia HUSM.**

Nama Pengkaji : Dr Ahmad Hadif Zaidin B Samsudin (Nombor MMC: 43414)

Penyelia : Dr Juhara B Harun (Nombor MMC: 34234)

Penyelia bersama : Prof Madya Dr Ariffin Nasir (Nombor MMC: 31850)

Penyelia bersama : Dr Rosnah Bahar (MMC Registration Number: 22368)

Pengenalan

Anda/ anak anda dipelawa untuk menyertai satu kajian penyelidikan secara sukarela yang melibatkan ujian pemeriksaan ultrasound kedua-dua belah leher untuk merekodkan bacaan ketebalan dinding salur arteri carotid anda/ anak anda. Bacaan ketebalan ini secara amnya dapat memberi maklumat tentang risiko penyakit sesalur darah tersumbat. Sebelum anda bersetuju untuk menyertai kajian penyelidikan ini, adalah penting anda membaca dan memahami borang ini. Sekiranya anda menyertai kajian ini, anda akan menerima satu salinan borang ini untuk disimpan sebagai rekod anda.

Tujuan kajian

Kajian ini bertujan untuk mengukur dan membandingkan ketebalan 'carotid intima media' (CIM) diantara pesakit thalassemia dengan populasi umum dengan menggunakan mesin ultrasound. Kajian terdahulu mendapati ukuran CIM yang tebal telah dikaitkan dengan risiko pembuluh darah tersumbat. Faktor- faktor seperti umur pesakit thalassemia, jangkamasa penyakit, bilangan pemindahan darah dan paras ferritin darah akan dikaji sekiranya mempunyai kaitan dengan ketebalan CIM. Ini supaya rawatan pesakit thalassemia dapat dipertingkatkan pada masa hadapan.

Kelayakan penyertaan

Doktor yang bertangungjawab dalam kalian ini atau salah seorang daripada kakitangan kajian akan membincangkan dengan anda tentang kelayakan anda untuk menyertai kajian ini. Adalah penting untuk anda berterus terang dengan doktor dan kakitangan tersebut tentang sejarah kesihatan anda.

Syarat kelayakan bagi menyertai kajian ini adalah:

Bagi pesakit thalassemia : anda telah/ sedang mendapat rawatan di HUSM
Bagi populasi umum : tiada

Anda tidak boleh menyertai kajian ini sekiranya mempunyai ciri-ciri berikut:

1. Mempunyai masalah tekanan darah tinggi, kencing manis, paras cholesterol tinggi, penyakit “connective tissue” atau metabolik.
2. Anda seorang perokok.
3. Anda sedang mengambil ubat anti- koagulant (cair darah) atau pil perancang kehamilan.
4. Anda terpaksa terlantar diatas katil pada jangkamasa yang lama.
5. Anda pernah mengalami kecederaaan kepada salur darah arteri karotid.

Prosedur kajian

Jika anda bersetuju membenarkan diri anda/ anak anda untuk menyertai kajian ini, satu set soalan-selidik yang pendek akan diberikan. Jika anda/ anak anda layak, pemeriksaan ultrasound kedua-dua belah leher akan dilakukan. Jika anda penghidap penyakit thalassemia, anda akan diberikan satu lagi set soal-selidik yang pendek untuk mengetahui jangkamasa penyakit anda/ anak anda dan bilangan transfusi darah telah diambil. Maklumat mengenai paras ferittin darah akan diambil daripada rekod perubatan anda.

Langkah –langkah ujian ultrasound:

Anda/ anak anda akan dibaringkan diatas katil dan kepala akan didongakkan sedikit keatas. Anda/ anak anda akan diminta untuk meminimumkan pergerakan kepala sebanyak mana yang buleh semasa pemeriksaan ultrasound kedua- dua belah akan dilakukan. Pemeriksaan ini mungkin akan mengambil masa diantara 10 hingga 20 minit dan anda/ anak anda akan dibenarkan pulang selepas pemeriksaan tersebut

Risiko

Gelombang ultrasound adalah gelombang yang tidak beradiasi, jadi tiada risiko radiasi di dalam pemeriksaan ultrasound dan ia adalah selamat. Jika ada maklumat tambahan yang dijumpai semasa kajian ini berjalan yang boleh mengubah keputusan anda bagi menyertai kajian ini, anda akan diberitahu dengan kadar segera.

Melaporkan pengalaman kesihatan

Jika anda mengalami apa-apa kecederaan, kesan buruk, atau apa-apa pengalaman kesihatan yang luarbiasa sejurus selepas kajian ini, pastikan anda memberitahu jururawat atau Dr. Ahmad Hadif Zaidin B Samsudin [No. Pendaftaran Penuh Majlis Perubatan Malaysia:43414] di talian 09-7673468 atau 013-9814800 secepat mungkin. Anda boleh membuat panggilan pada bila-bila masa, siang atau malam, untuk melaporkan pengalaman sedemikian.

Penyertaan dalam kajian

Penyertaan anda dalam kajian ini adalah secara sukarela. Anda berhak menolak untuk menyertai kajian ini atau anda boleh menamatkan penyertaan anda pada bila-bila masa, tanpa sebarang hukuman atau kehilangan manfaat yang sepatutnya anda perolehi.

Penyertaan anda juga mungkin boleh diberhentikan oleh doktor yang terlibat dalam kajian ini tanpa persetujuan anda. Sekiranya anda berhenti menyertai kajian ini, doktor yang terlibat di dalam kajian ini atau salah seorang kakitangan akan berbincang dengan anda mengenai apa-apa isu perubatan berkenaan dengan pemberhentian penyertaan anda.

Manfaat yang mungkin

Prosedur kajian ini akan diberikan kepada anda tanpa kos. Anda mungkin menerima maklumat tentang kesihatan anda daripada pemeriksaan ultrasound dan ujian makmal yang dilakukan dalam kajian ini. Hasil atau maklumat kajian ini diharapkan, dapat memberi manfaat kepada pesakit-pesakit pada masa hadapan. Anda tidak akan menerima sebarang pampasan kerana menyertai kajian ini. Namun sebarang keperluan perjalanan berkaitan dengan penyertaan ini akan diberi.

Persoalan

Sekiranya anda mempunyai persoalan mengenai kajian ini atau berkenaan hak-hak anda, sila hubungi:

Dr Ahmad Hadif Zaidin b Samsudin (No MMC: 43414)
Jabatan Radiologi, HUSM
Pusat Pengajian Sains Perubatan
USM Kampus Kesihatan
16150 Kubang Kerian.
Tel 013-9814800.
Email: hadif@kk.usm.my/ sam_hadif@yahoo.com

Sekiranya anad mempunyai sebarang soalan berkaitan kelulusan etika kajian ini, sila hubungi:

En. Mohd Bazlan Hafidz Mukrim
Setiausaha Jawatankuasa Etika Penyelidikan (Manusia) USM
Pusat Inisiatif Penyelidikan -Sains Klinikal & Kesihatan
USM Kampus Kesihatan.
No. Tel: 09-767 2354 / 09-767 2362
Email : bazlan@usm.my/Jepem@kk.usm.my

Kerahsiaan

Maklumat perubatan anda akan dirahsiakan oleh doktor dan kakitangan kajian. Ianya tidak akan dedahkan secara umum melainkan jika ia dikehendaki oleh undang-undang.

Data yang diperolehi dari kajian yang tidak mengenalpasti anda secara perseorangan mungkin akan diterbitkan untuk tujuan memberi pengetahuan baru.
Rekod perubatan anda yang asal mungkin akan dilihat oleh pihak penyelidik, Lembaga Etika kajian ini dan pihak berkuasa regulatori untuk tujuan mengesahkan prosedur dan/atau data kajian klinikal. Maklumat perubatan anda mungkin akan disimpan dalam komputer dan diproses dengannya.
Dengan menandatangani borang persetujuan ini, anda membenarkan penelitian rekod, penyimpanan maklumat dan pemindahan data seperti yang dihuraikan di atas.

Tandatangan

Untuk dimasukkan ke dalam kajian ini, anda atau wakil sah anda mesti menandatangani serta mencatatkan tarikh halaman tandatangan.

Borang Keizinan Pesakit
(Halaman Tandatangan)

Tajuk Kajian*:* **Kajian tentang ketebalan 'carotid intima media' di kalangan pesakit thalassemia HUSM.**

Nama Pengkaji : Dr Ahmad Hadif Zaidin B Samsudin (Nombor MMC: 43414)

Penyelia : Dr Juhara B Harun (Nombor MMC: 34234)

Penyelia bersama : Prof Madya Dr Ariffin Nasir (Nombor MMC: 31850)

: Dr Rosnah Bahar (MMC Registration Number: 22368)

Untuk menyertai kajian ini, anda atau wakil sah anda mesti menandatangani mukasurat ini. Dengan menandatangani mukasurat ini, saya mengesahkan yang berikut:

- Saya telah membaca semua maklumat dalam Borang Maklumat dan Keizinan Pesakit ini termasuk apa-apa maklumat berkaitan risiko yang ada dalam kajian dan saya telah pun diberi masa yang mencukupi untuk mempertimbangkan maklumat tersebut.
- Semua soalan-soalan saya telah dijawab dengan memuaskan.
- Saya, secara sukarela, bersetuju menyertai kajian penyelidikan ini, mematuhi segala prosedur kajian dan memberi maklumat yang diperlukan kepada doktor, para jururawat dan juga kakitangan lain yang berkaitan apabila diminta.
- Saya boleh menamatkan penyertaan saya dalam kajian ini pada bila-bila masa.
- Saya telah pun menerima satu salinan Borang Maklumat dan Keizinan Pesakit untuk simpanan peribadi saya.

Nama Pesakit (Dicetak atau Ditaip)	Nama Singkatan & No. Pesakit
No. Kad Pengenalan Pesakit (Baru)	No. K/P (Lama)
Tandatangan Pesakit atau Wakil Sah	Tarikh (dd/MM/yy)
Nama & Tandatangan Individu yang Mengendalikan Perbincangan Keizinan (Dicetak atau Ditaip)	Tarikh (dd/MM/yy)
Nama Saksi dan Tandatangan	Tarikh (dd/MM/yy)

Nota: **i) Semua subjek/pesakit yang mengambil bahagian dalam projek penyelidikan ini <u>tidak dilindungi insuran</u>.**

Appendix 3

Borang kaji-selidik subjek A (Questionnaire A)

No pendafaran:_____

Sila jawab soalan dibawah dengan sejujur yang buleh. Segala maklumat yang diberikan adalah rahsia dan hanya akan digunakan untk tujuan penyelidikan sahaja.

1.	Adakah anda/ anak anda mengidap penyakit seperti dibawah:		
	Darah tinggi	Ya	Tidak
	Kencing Manis	Ya	Tidak
	Hypercholesterolemia/ Tinggi cholesterol	Ya	Tidak
	Obesiti/ berat berlebihan	Ya	Tidak
	Penyakit kronik (penyakit metabolik, penyakit “connective tissue”)	Ya	Tidak

2.	Adakah anda/ anak anda menghisap rokok atau sedang mengambil ubat anti- koagulant (cair darah) atau pil perancang kehamilan	Ya	Tidak

3	Pernakah sebelum ini anda/ anak anda ditimpa sakit yang teruk sehingga tidak dapat bangun dan terpaksa terlantar diatas katil pada jangkamasa yang lama.	Ya	Tidak

4	Pernakah sebelum ini anda/anak anda mengalami kecederaaan kepada salur darah arteri carotid (leher).	Ya	Tidak

Appendix 4

Borang kaji-selidik subjek B (Questionnaire B)

No pendafaran:_____

1.	Berapakah umur anda/ anak anda	……..tahun

2.	Adakah anda/ anak anda mengidap penyakit thalassemia - Jika ya, sila teruskan menjawab soalan seterusnya	Ya	Tidak

3.	Sejak umur berapa tahun anda/ anak anda mengidap penyakit thalassemia?	……..tahun

4.	Sudah berapa kali anda/ anak anda telah menerima transfusi darah	……..kali

Data collection sheet

General population

Registered number (RN)	CIMT 1st reading	CIMT 2nd reading	CIMT 3rd reading	CIMT mean
1.	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	……..mm
2.	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	……..mm
3.	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	……..mm
4.	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	……..mm
5.	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	……..mm
6.	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	……..mm
7.	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	……..mm

Data collection sheet

Thalassemia subjects

Registered number (RN)	CIMT 1st reading	CIMT 2nd reading	CIMT 3rd reading	CIMT mean
	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	Right=…...mm Left=…….mm	……..mm

Age : _____ year

Disease duration : _____ year

Number of blood transfusion : _____ times

Serum ferittin level : _____ mg/dL