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.

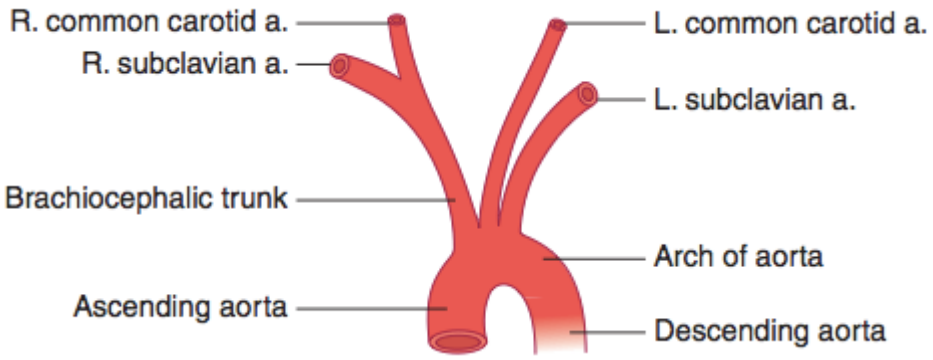

Figure 2.1: Moss common appearance of aortic arch. (Adapted from Butler et al., 2012)

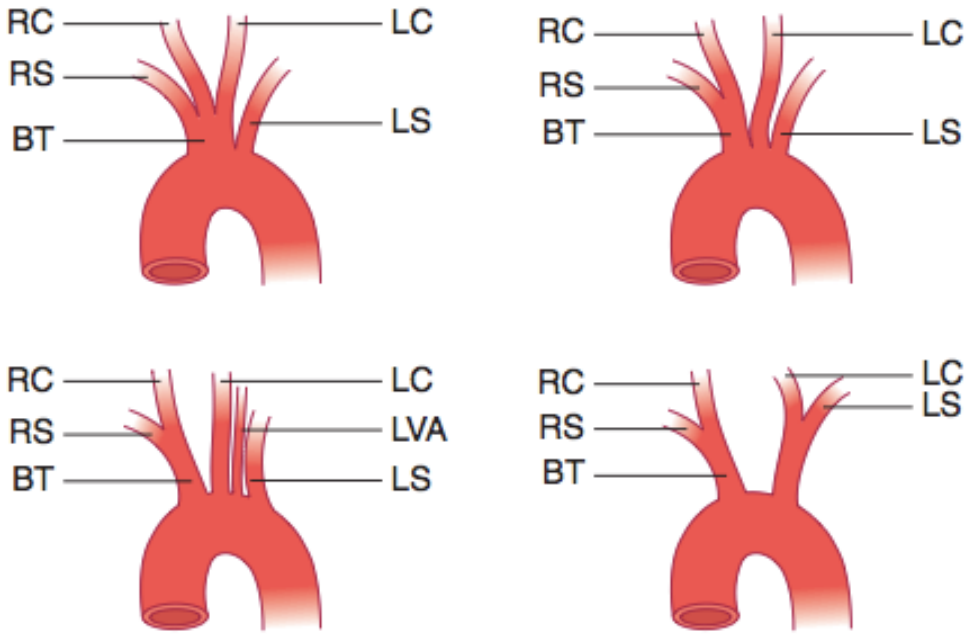

Figure 2.2: Normal variant of the aortic arch and origin of the common carotid artery. Key: RS- right subclavian artery, RC- right common carotid artery, BT – brachiocephalic trunk LC- left common carotid artery, LS- left subclavian artery and LVA- left vertebral artery. (Adapted from Butler et al.,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

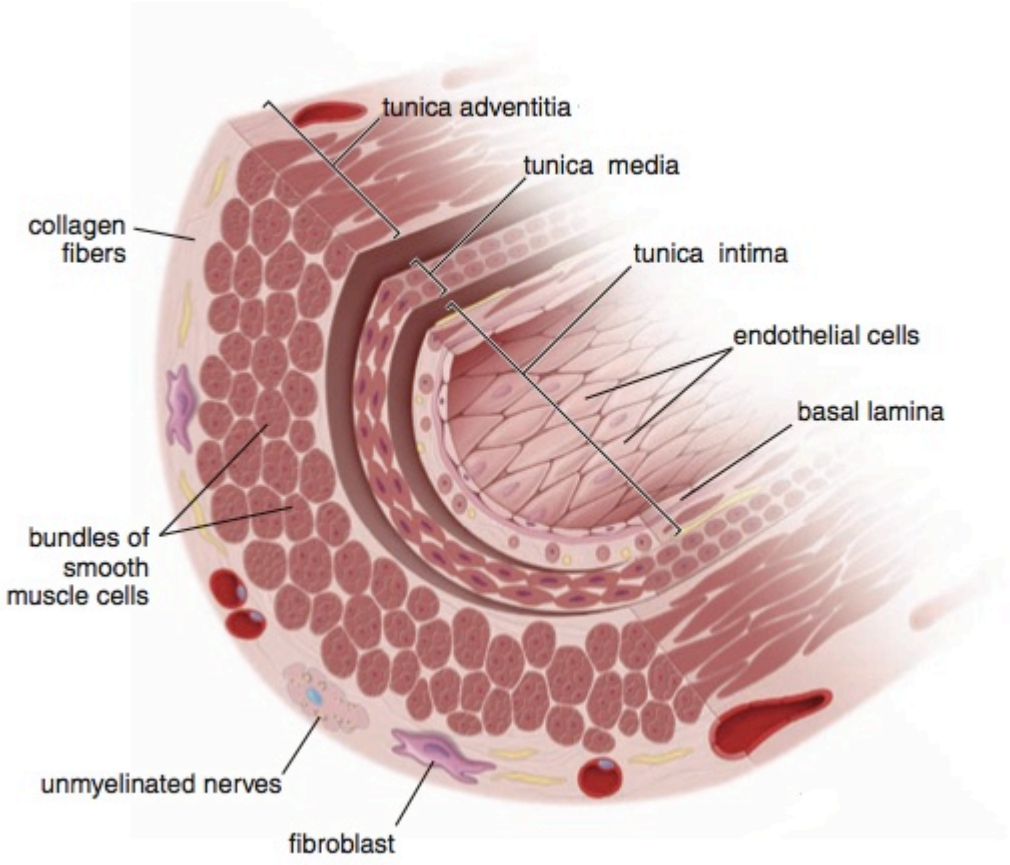

Figure 2.3: Normal arterial wall structure. (Adapted from Pawlina and Ross, 2011)

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.

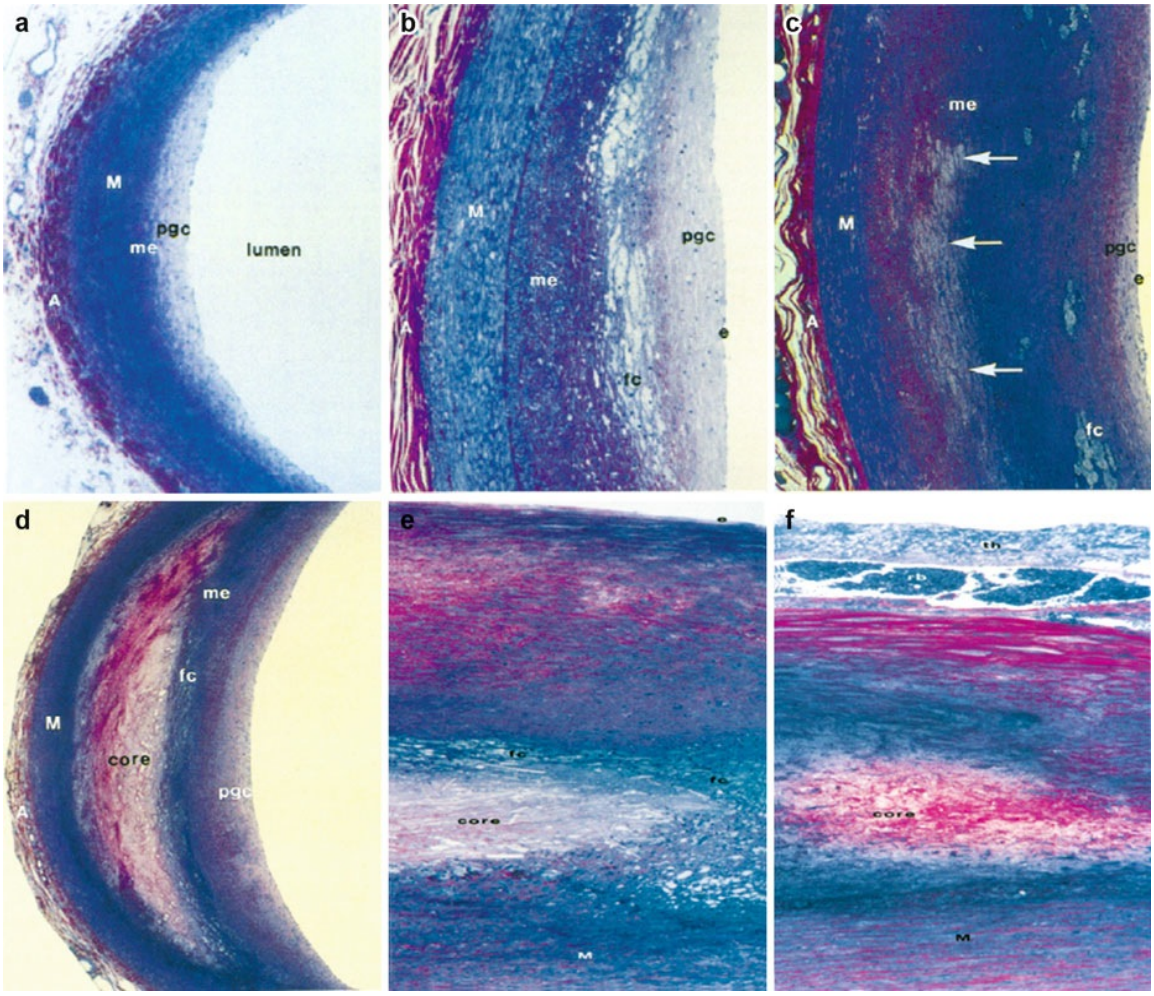

Figure 2.4: Histological examples of atherosclerotic plaque types classified according to the American Heart Association criteria (Adapted from Nicolaides *et al.*, 2011).

(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.

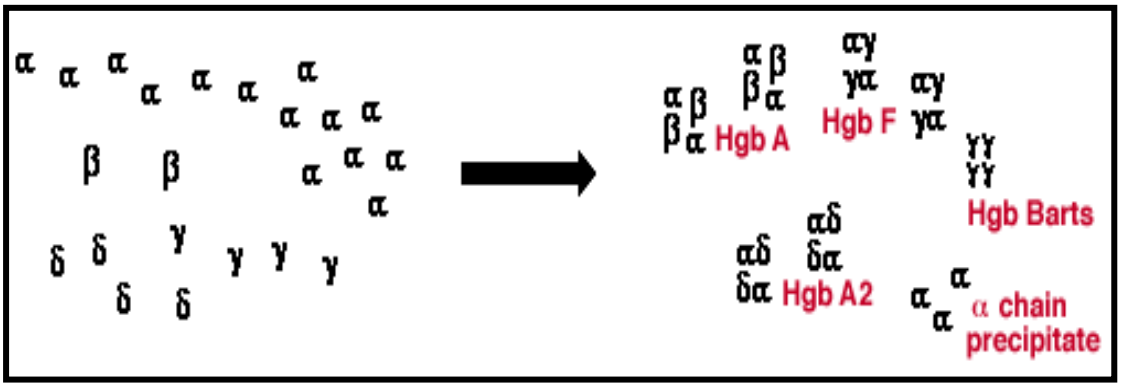

Figure 2.5: Type of beta thalassemia. (Source: http://www.med-ed.virginia.edu/courses/path/innes/rcd/thalassemia.cfm)

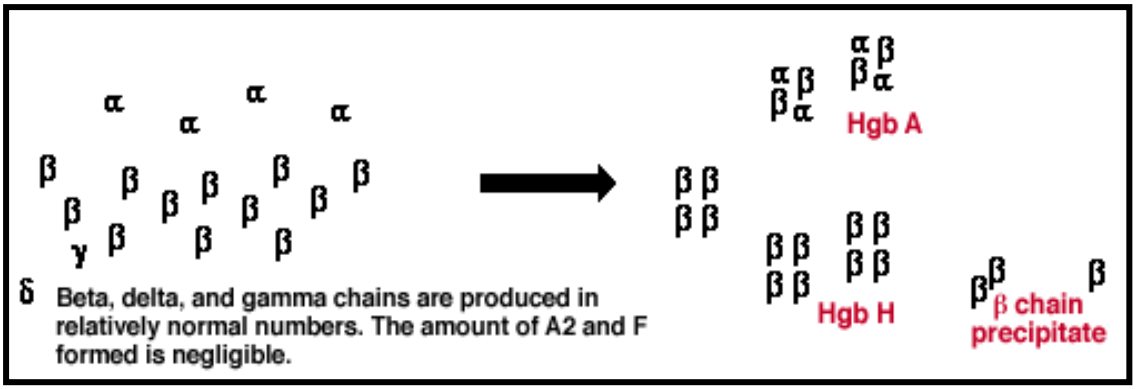

Figure 2.6: Type of alpha thalassemia. (Source: http://www.med-ed.virginia.edu/courses/path/innes/rcd/thalassemia.cfm)

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%),