DETECTION ON COMMON DELETIONAL ALPHA THALASSAEMIA IN PREGNANT WOMEN BY POLYMERASE CHAIN REACTION TECHNIQUES

by

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Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

May 2015

ACKNOWLEDGEMENT

First of all, I would like to thank Allah (S.W) for the healthiness and giving me the strength and courage to persevere throughout the duration of this research project and made all of this and everything possible.

I would like to acknowledge Universiti Sains Malaysia (USM) for the project grant (1001/PPSP/812003) and Ministry of Science, Technology and Innovation (MOSTI) for awarding me National Science Fellowship (NSF). It is a pleasure to thanks those who made this thesis possible especially my supervisors, Professor Dr. Rosline Hassan and my co-supervisors PM Dr Rosnah Bahar and Dr Noraliza Abd Ghaffar. I am heartily thankful to them for their encouragement, supervision and support from the beginning to the final of my Master research.

I also want to deliver my appreciation to the entire patient involved in my study, staff nurses at Obstetrics and Gynaecology Clinics, HUSM, Pn. Noor Aini and Pn. Che Saagah who help me in recruiting the blood samples and Cik Selamah for troubleshooting laboratory work problems. I would like to thanks all the staff at Haematology Department, Transfusion Medical Unit and Obstetrics and Gynaecology Clinics for their helps and cooperation.

I also would like to show my gratitude to my friends: Nur Hazyyah Hassan, Nik Mohd Khuzaimi Nik Man, Mohd Annuar Nordin, Mohd Adzha Majid, Imilia Ismail, Siti Asma Mat Jusoh, Diana Alias, Pn Marianor Mahat, Adhia Razak,

Siti Nur ‘Asyiqin Sabaruddin, Nur Hasnah Ma’amor, Munira Mohamad, Norazwana Zakaria, Siti Nurul Syuhada, Khaizil Emylia Zazali, Firdaus Mat Isa, Che Wan Salma, Noor Aini Sudin, Nur Liyana and others for being such great supportive friends.

My special thanks to my beloved husband Mohd Fakrulnizam Md Sahak, my father Abdullah Bin Awang and my late mother Mek Zah Nawang. Last but not least, thanks to all who contributed in this study directly or indirectly. May Allah bless all of you.

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ARMS	Amplification-refractory mutation system
ASO	Allele-specific oligonucleotide
Bam HI	*Bacillus amyloliquefaciens*
Bgl II	*Bacillus globigii*
C	Celsius
CE	Capillary Electrophoresis
dATP	deoxyadenosine triphosphate
DCIP	dichlorophenol-indolphenol
dCTP	deoxycytosine triphosphate
ddH2O	distilled deionized water
dGTP	deoxyguanine triphosphate
dH2O	distilled water
DNA	Deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
dTTP	deoxythymine triphosphate
E.coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
FBC	Full blood count
--FIL	Filipino
fL	femtoliter
H20	water
Hb	Haemoglobin

Hb A2	Haemoglobin A2
Hb E	Haemoglobin E
Hb F	Haemoglobin F
Hb H	Haemoglobin H
Hb E/ß	Haemoglobin E β
Hb E/α	Haemoglobin E α
Hct	Haematocrit
HPLC	High performance liquid chromatography
HUSM	Hospital Universiti Sains Malaysia
kb	Kilo base pair
LCD	liquid-crystal display
M	Marker
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
$--^{MED}$	Mediterranean
MgCl2	Magnesium chloride
NaOH	Sodium hydroxide
NTC	No template control
OF	Osmotic fragility
PCR	Polymerase Chain Reaction
pg	Picograms
QC	Quality control
RBC	Red blood cell
RDW	Red blood cell distribution width

ROC	Receiver operating characteristic
SD	Standard deviation
SDW	Sterile distilled water
$--^{SEA}$	Southeast Asian
SPSS	Statistical Package for Social Sciences
TBE	tris-borate-EDTA
$--^{THAI}$	Thailand
U	Unit
UMMC	Universiti Malaya Medical Centre
USM	Universiti Sains Malaysia
UTR	Untranslated region
UV	Ultra-violet
α	alpha
α^{Th}	non deletional α-thalassaemia mutation involving either α2- or α1-globin gene

DETECTION ON COMMON DELETIONAL ALPHA THALASSAEMIA IN PREGNANT WOMEN BY POLYMERASE CHAIN REACTION TECHNIQUES

ABSTRACT

Thalassaemia is the most common inherited disorder worldwide and represent as a major health problem in many areas and approximately 4.5%-6% of Malaysians are carrier of this genetic disorder. There are two type of thalassaemia which α and β thalassemia. α-thalassaemia either the deletion of a single or double α-globin gene deletion that is located at position 16p3.3 is the one of the most common genetic disorder in the world. In Malaysia, the incidence is 4.5%. The aims of this study were to identify and characterised the common deletional type cases of α-thalassaemia in Malay pregnant women at HUSM by molecular method. A total of 200 Malay pregnant women who attended for an antenatal check-up at Hospital Universiti Sains Malaysia were screened for α-thalassaemia. DNA was extracted from 200 pregnant women blood using commercial DNA extraction kit prior to PCR amplification. Of these, 16 were excluded as they were diagnosed as β-thalassaemia/Hb E trait. Out of 184 genomic DNA, 17 (9.2%) were possessed α-thalassaemia deletion. The genotype could be identified to -$\alpha^{3.7}$/αα in 15 (8.1%) and --^{SEA}/αα in 2(1.1%). While -$\alpha^{4.2}$ kb deletion and --^{THAI} deletion was not detected in our subjects. Thus, the most common deletion in the Malays pregnant women were -$\alpha^{3.7}$ followed by --$^{SEA.}$ The molecular method has been established to detect these carriers. The presence of two

gene deletion evidenced by $--^{SEA.}$ showed the importance to screen α-thalassaemia among Malay pregnant women and subsequent screening patients' spouse to exclude hydrops fetalis. Detection of $--^{SEA}$ α-thalassaemia by PCR techniques is convenient, and suitable to be used as a confirmatory test.

PENGESANAN PEMOTONGAN UMUM TALASEMIA ALFA DALAM WANITA MENGANDUNG YANG DITENTUKAN OLEH TEKNIK TINDAKBALAS BERANTAI POLIMERASE (PCR)

ABSTRAK

Talasemia adalah penyakit warisan yang paling umum di seluruh dunia dan merupakan masalah kesihatan utama dan kira-kira 4.5% -6 % daripada rakyat Malaysia adalah pembawa penyakit genetik ini. Talasemia terbahagi kepada dua jenis iaitu α-talasemia dan β-talasemia. α-talasemia sama ada pemotongan pada gen α-globin tunggal atau berganda yang terletak di kedudukan kromosom 16p3.3 adalah merupakan salah satu penyakit genetik yang paling umum di dunia. Kira-kira 4.5% pengidap penyakit α-talasemia di Malaysia. Tujuan kajian ini adalah untuk mengenal pasti jenis pemotongan α-talasemia yang umum di kalangan wanita hamil Melayu di HUSM menggunakan teknik molekul. Seramai 200 orang wanita Melayu hamil yang hadir untuk pemeriksaan sebelum bersalin di Hospital Universiti Sains Malaysia telah disaring untuk α-talasemia. DNA telah diektrak daripada darah 200 orang wanita Melayu hamil tersebut dengan menggunakan kit ekstrak darah komersial sebelum diamplifikasikan melalui kaedah tindakbalas berantai polimerase (PCR). Daripada jumlah ini, sebanyak 16 orang telah dikecualikan kerana mereka disahkan mengidap β-talasemia atau Hb E. Daripada 184 DNA genomik, 17 (9.2%) telah mempunyai pemotongan α-talasemia. Genotip yang dikenalpasti adalah -

$\alpha^{3.7}$/αα seramai 15 orang (8.1 %) dan --^{SEA}/αα seramai 2 orang (1.1%). Walaubagaimanapun, pemotongan jenis -$\alpha^{4.2}$ dan --^{THAI} tidak berjaya dikesan. Oleh itu, jenis pemotongan yang paling umum di kalangan wanita Melayu hamil adalah -$\alpha^{3.7}$ diikuti oleh --^{SEA}. Teknik PCR telah berjaya di tubuhkan. Jenis pemotongan dua gen atau pemotongan berganda di sahkan dengan kehadiran --^{SEA} amat penting dikenalpasti terutama dalam wanita Melayu yang hamil supaya saringan penyakit α-talasemia dapat dijalankan keatas suami pesakit tersebut bagi menghalang kejadian hidrop fetalis. Penemuan --^{SEA} menggunakan teknik PCR merupakan teknik yang sesuai digunakan dalam pengesahan penyakit α-talasemia.

CHAPTER 1

INTRODUCTION

1.1 Statement and Significance of the problem

Thalassaemia is a major health problem worldwide and approximately 1 in 14 is carrier (Xiofeng, G. and Yitao, Z., 2002). It is common in people of Asian descent and has emerged as public health problem in Malaysia. In Kelantan, the Northeast region of Malaysia, which is situated at the border of Thailand, thalassaemia is prevalent. It is expected the disease phenotype and genotype would resemble that of Thailand. In Thailand, with the total of 60 million populations, approximately one percent affected individuals and more than 20 million were thalassaemia carriers (Greenberg *et al*., 2001).

A preliminary study done among blood donor in USM showed that the prevalence of thalassaemia was 15% with Hb E/α is the commonest followed by Hb E trait (Rosline, H *et al*., 2006). In another local data, the commonest thalassaemia among transfusion dependent patients is Hb E/ß. These data shows an extreme diversity in clinical presentation of thalassaemia patients spanning from asymptomatic thalassaemia trait to transfusion dependent (Rozitah, R *et al*., 2008). Population screening for thalassaemia carrier is first

needed to understand the genotype basis of this complex interaction which gives rise to the major socioeconomic problem to the country.

The α-thalassaemia is mainly caused by a large deletion of the α-globin gene and occurs when one or more of the four alphas (α) chain genes fail to function. α-thalassaemia is the one of the most common inherited disorder of haemoglobin synthesis and commonly found in Southeast Asian, Mediterranean and Middle Eastern population (Guvenc, B *et al*, 2010). Clinical phenotype of the carriers varies according to the number of affected gene.

α-thalassaemia can be classified into two types which were α-thalassaemia-1 (α^0-thalassaemia), the deletion of both α1- and α2- globin genes and this type have mild microcytic, hypochromic anaemia with normal haemoglobin A_2 levels and α-thalassaemia-2 (α^+-thalassaemia) where only one α-globin gene deletion has occurred, present with no detectable red blood cell abnormalities or globin chain imbalance (Weatherall, D.J., and Clegg, J.B., 2001).

The prevalence of α-thalassaemia-1 is around 3-4% and for α-thalassaemia-2 is 20-30% (Sanguansermsri, T., *et al*, 1999) and it resulted from deletion and non-deletional mutations. The commonest form is α-gene deletion. The five common type of α-gene deletion in South East Asian region are $--^{SEA}$, $--^{THAI}$,$--^{FIL}$,$-\alpha^{3.7}$ and $-\alpha^{4.2}$ (D.R. Higgs, 2013). The worst outcome of

α-thalassaemia is Hb Bart's hydrops foetalis. However, Hb H disease due to three deletions of genes is associated with increased morbidity.

Since thalassaemia is now a public health problem in Malaysia, there is need to raise public awareness in the community. This study focused on detection of carrier of the commonest α-gene deletion which $--^{SEA}$, $--^{THAI}$, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ among pregnant women in this population. $--^{SEA}$ deletion is severe form of α-thalassaemia determinant in Southeast Asian country including Malaysia.

The control of thalassaemia remains a major challenge. The conventional approach to screen and diagnose haemoglobinopathies requires a combination of test. These methods, including erythrocyte indices and morphology, Hb electrophoresis, quantitation of Hb A_2, Hb E, and Hb F, and detection of erythrocytes containing Hb H inclusion bodies (Sanchasuriya, K *et al*., 2003). To screen for thalassaemia carriers in the whole population, is costly however it has clinical importance.

Among our population, pregnant women is the group of choice for the screening strategy in order to characterised their carrier state and to provide sufficient information beside to estimate the risk that their children would have severe disease such as α-globin mutation and also β-globin mutation. All the strategies are aiming at reducing the birth of dependent form of thalassaemia that can increase public health problem in Malaysia.

In order to succeed in prevention and control of thalassaemia, we have develop a diagnostic tool that is polymerase chain reaction (PCR) techniques to detect the most common form of α-thalassemia such as $--^{SEA}$ deletion, $--^{THAI}$ deletion, $-\alpha^{3.7}$ rightward deletion and $-\alpha^{4.2}$ leftward deletion in Malay population. This method is simple, reliable and suitable for population screening and routine diagnosis. Based on this information, genetic counselling can be practiced later.

1.2 Literature Review

1.2.1 Haemoglobin Structure and Function

Haemoglobin is an iron-rich protein in red blood cells. Haemoglobin is essential for the existence of human life and also the remarkable protein that enables red blood cells to carry oxygen and carbon dioxide. Haemoglobin tetramers are comprised of the four subunits, two α-globin chains and two β-globin chains and each having one polypeptide chain and one haem group (Alain, J., 2006)(Figure 1.1). All of which take the form of α helices.

In thalassaemia disorders, the haem part of haemoglobin is entirely normal. The defect lies exclusively with the globin part of haemoglobin. This defect results in the underproduction of globin and, hence, the underproduction of haemoglobin.

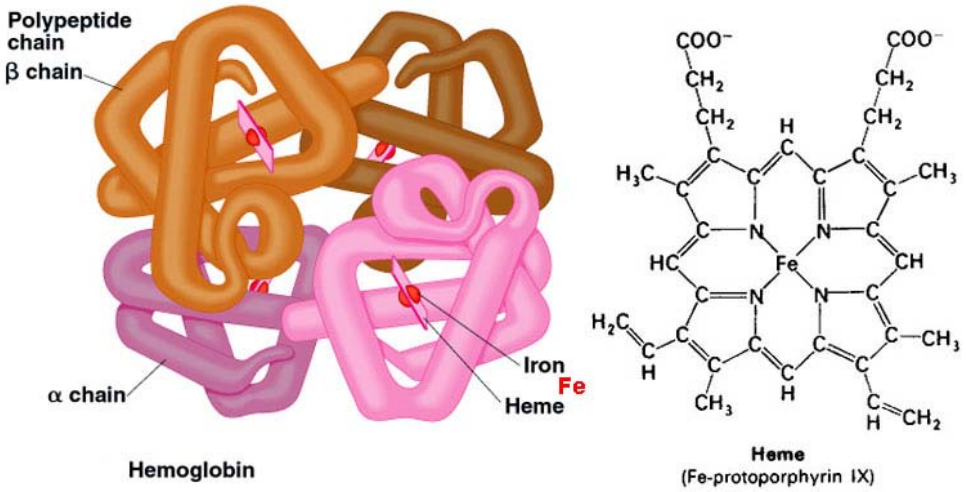

Figure 1.1 Structure of the haemoglobin molecule with four globin chain and a haem group within each.
(Adapted from www.themedicalbiochemistrypage.org/protein-structure.php).

1.2.2 Haemoglobin Synthesis during Development

Normal adult have major haemoglobin called Hb A comprising about 97% of the total and a minor component, Hb A_2 that accounts 2-3% of total haemoglobin in foetus. Foetal haemoglobin, Hb F ($\alpha_2\gamma_2$) represents 90-95% of haemoglobin by 34-36 week gestation. After 34 week gestation, Hb A production increase significantly as falls of Hb F productions. Small amount of Hb A_2 is produced from birth and usually reached adult levels by 6 months of age, although it can rise further for the first 1-2 years of life (Ryan K, *et al*, 2010).

During the embryonic stages of foetal development, there are three embryonic haemoglobin, haemoglobins Gower 1, Gower 2 and haemoglobins Portland (Table 1.1). The production of this different haemoglobin is a reflection of a series of physiological adaptations to differing O_2 requirement at various stages of development (Ryan K, *et al*, 2010). The normal pattern of haemoglobin synthesis is summarised in Figure 1.2

Table 1.1: Haemoglobin gene expression

Developmental Period	**Haemoglobin Species**	**Globin Chain**
Embryonic	Gower 1	$\zeta_2 \varepsilon_2$
	Portland	$\zeta_2 \gamma^{G \text{ or } A}$
	Gower 2	$\alpha_2 \varepsilon_2$
Foetal	Haemoglobin F	$\alpha_2 \gamma_2^{G \text{ or } A}$
Adult	Haemoglobin A	$\alpha_2 \beta_2$
	Haemoglobin A_2	$\alpha_2 \delta_2$
	Haemoglobin F	$\alpha_2 \gamma_2^{G \text{ or } A}$

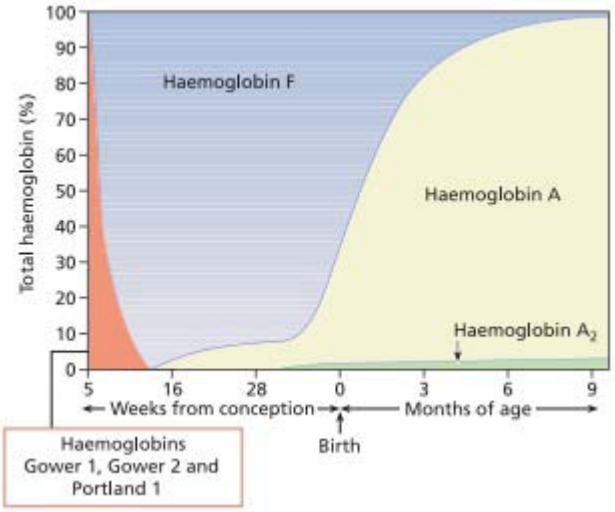

Figure 1.2: The normal pattern of haemoglobin synthesis. (Adapted from Ryan K, *et al*, 2010).

1.2.3 Thalassaemia

The name of thalassaemia is derived from a combination of two Greek words which are thalassa and anaemia. Thalassa means the Mediterranean Sea and anaemia in Greek means weak (an) and blood (haima). Thalassaemia is a group of genetic, inherited disorders of the blood. More specifically, it is a disorder of the haemoglobin molecule inside the red blood cells. It is an inherited genetic disease where it is passes from parents to children through the genes. It is not infectious and cannot be passed from one individual to the other by personal or any other contact. Thalassaemia occurs widely throughout the Mediterranean region, Africa, Indian, Middle East and Southeast Asia population. The most important disorders are α-thalassaemia and β-thalassaemia (Rachmilewitz, E. A., and Giardina, P.J., 2011).

α-thalassaemia is caused by the deletion or mutation of α-globin gene and characterised by reduce or absence of the α-globin chain (Rappaport V.J., *et al*, 2004). In contrast, β-thalassaemia is due to a point mutation in one of the β-globin gene and leading to decreased or absence of β-globin chain of the haemoglobin (Mirbehbahani, N.B., *et al*, 2013).

1.2.4 Molecular basis of α-thalassaemia

1.2.4.1 Alpha Globin Gene Cluster

The genes that regulate the synthesis and structures of the different globins are organised in two separate clusters. The α-globin gene cluster is located on chromosome 16 at position 16p13.3 and mutations or deletions affecting either one or more α-globin genes results in α-thalassaemia syndrome. In contrast, the β-globin gene cluster is located on chromosome 11. The α-globin gene cluster contains one embryonic ζ- and two α-globin gene designated α1 and α2 arranged in the order of 5'- ζ2-α2-α1-3' on each chromosome 16 (Plate 1.1). There are four pseudogenes: ψζ1, ψα2, ψα1 and ψθ1 within the α -globin gene cluster (Forget, B.G., 2001). Since individual has two chromosomes 16, there are usually a total of four functional α-globin genes. But the number can vary from none to as many as eight α-globin genes due to misaligned recombination (David, H.K. Chui, 2005).

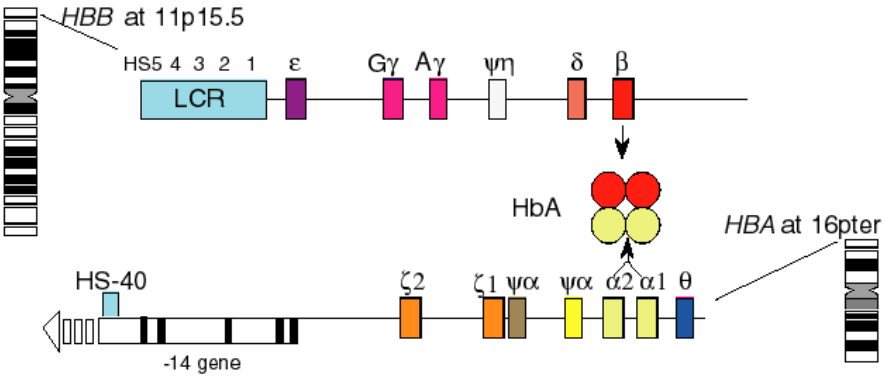

Plate 1.1: Schematic representation of the α-globin gene cluster and β-globin gene cluster. The β-LCR and HS40 is the main regulatory region for these gene clusters. (According to Weatherall & Clegg, 2001)

1.2.5 α-Thalassaemia

α-thalassaemia is classified into deletional and non-deletional types (Bain BJ, 2006). There are at least 40 different deletions in deletional mutation and the size of the deletion is important and affects the clinical phenotype of hydrops foetalis. In contrast, non-deletional mutations may have a more severe phenotype than most of the deletional mutations. The most common non-deletional α-thalassaemia mutation is Haemoglobin Constant Spring (Vichinsky, E.P., 2009).

α-thalassaemia is mainly caused by a large deletion of the α-globin gene (Choopayak, C *et al*., 2005). The α-thalassaemia is a haemolytic anaemia resulting from deficient synthesis of α-globin. Normal individuals have four α-globin genes. α-thalassaemia can results from deletion of either one, two, three or four α-globin genes (Wee, Y.C *et al*., 2005). α-thalassaemia also can cause the progressive decrease in α-globin chain synthesis in carrier of α-thalassaemia, haemoglobin H disease and Haemoglobin Bart's hydrop foetalis syndrome (Winichagoon, P *et al*, 1984).

There are two major types of α-thalassaemia determinants which α° and α^{+}. α°-thalassaemia or formerly called as α-thalassaemia-1 represent a condition in which both α-globin genes on chromosome are inactivated. In contrast, α^{+}-thalassaemia also called as α-thalassaemia-2 represent a condition in which one of the two linked α-globin genes on a chromosome is inactivate

and can be caused either by deletion or mutation (Neishabury, M *et al*, 2001).

1.2.5.1 Common α-thalassaemia deletions

(a) α°-thalassaemia or α-thalassaemia-1

α°-thalassaemia deletion determinant caused by complete or partial deletion of both genes in cis. Generally there are about 20 α°-thalassaemia deletions that tend to be recurrent in various populations. The prevalence of α°-thalassaemia in Columbia is 3-6% with $--^{SEA}$/αα genotype (Bergstrome Jones, A.K., and Poon, A., 2002). In pregnant Lao women, 8.7% of SEA deletion were detected (Savongsi, O., 2008). In Malaysia itself from the data by Institute for Medical Research showed 17.6% of having $--^{SEA}$ deletion. (Rahimah, A *et al*, 2013)

In South East Asia population, the most common α°-thalassaemia is the Southeast Asian ($--^{SEA}$) deletion, Filipino ($--^{FIL}$) and the Thai ($--^{THAI}$) deletion, respectively (Wee, Y.C *et al* 2005; Nattaya, Sae-Ung, 2007; Chiara, D.B *et al*, 2006). While, the Mediterranean ($--^{MED}$) and 20.5 kb deletion ($--^{20.5}$) was commonly found in Mediterranean populations. These deletions remove both functional α-globin genes but spare the ζ2-globin gene intact (Nicholls, R.D *et al*, 1987). The α°-thalassaemia is the complete loss of both functional α-globin genes in tandem on the same chromosome mainly caused by deletion

and the deletions presented with mild hypochromic microcytic anaemia (Rahimah, A *et al*, 2012).

In α°-thalassaemia, there is a deletion that removes about 17.5kb of DNA. This includes the α1 and α2 genes from the α-globin gene cluster. The 5' breakpoint may start within the third exon of the ¥£ gene and the 3' end may terminate within the hypervariable region which was located at the 3' end of the α-globin gene complex (Fuchareon, S., & Winichagoon. P., 2002).

(b) α^{+}-thalassaemia or α-thalassaemia-2

Deletion of either one of the linked pairs of α-globin gene is classified as α^{+}-thalassaemia. α^{+}-thalassaemia is the most common α-thalassaemia mutation. There are two common deletion forms of α^{+}-thalassaemia that are designated by the size of the deletion as $-\alpha^{3.7}$ and $-\alpha^{4.2}$. The most common form is the rightward $-\alpha^{3.7}$ deletion which involving a deletion of 3.7kb of DNA between the duplicate α-gene, while the less common deletion involving a deletion of 4.2kb of DNA including the α2 gene deletion are the leftward $-\alpha^{4.2}$ deletion. Both of these deletions result from unequal homologous recombination within the α-globin cluster (Fuchareon, S., & Winichagoon, P., 2002).

Each α-globin gene is located within a homologous area of 4 kb length including two non-homologous sections. These two globin genes are imbedded in a large region of homology which is dividing by subsequent

insertions and deletions to give three homology sub segments on each α-globin gene. These homology sub segments are called X, Y and Z boxes. Duplicated or the crossover can occur between the two X regions separated by 4.2kb or the two Z regions which separated by 3.7kb apart (Neishabury, M *et al*, 2001).

There are over 20 million carrier of α^{+}-thalassaemia in the world, with the highest incidence found in the population of India, Southeast Asian and Africa and less commonly in the Mediterranean and Middle East. The $-\alpha^{3.7}$ deletion is the extremely wide spread. The frequency of the $-\alpha^{3.7}$ mutation can reach very high level. In Malaysia, the prevalence of the heterozygous state of $-\alpha^{3.7}$ is approximately 5-10% and $-\alpha^{4.2}$ were 0.06% (Wee, Y.C *et al*, 2005), in the Mediterranean region, 43% of Jordanian thalassemic represents the $-\alpha^{3.7}$ deletion (Abu Ghoush, 2008). While the $-\alpha^{4.2}$ is frequently found in Asia as in Malaysia, in the previous study done by Wee, Y.C, 0.06% of $-\alpha^{4.2}$ deletion was detected among randomly selected 650 pregnant women in University Malaya Medical Centre (UMMC).

1.2.5.2 Clinical Syndromes of α - thalassaemia

The clinical syndromes of α-thalassaemia can be subdivided into four categories which are α-thalassaemia trait, silent carrier, Hb H disease, and Haemoglobin Bart's hydrops foetalis syndrome.

- Silent Carrier (loss of one α globin gene). The haematological parameters are within normal limit.
- α-thalassaemia trait (loss of two α globin gene). There a mild haematological changes with normal or borderline low Hb level, low MCV but no major clinical abnormality.
- Hb H disease (loss of three α globin gene). The clinical severity is considerable variability.
- Haemoglobin Bart's hydrops foetalis syndrome (no functional α-gene). Loss of all four α globin gene showed severe anaemia and hypoxia in utero, incompatible with life, resulting in mid- to late-gestational stillbirth of hydropic foetus.

(a) Silent carrier: Loss of a single α-globin gene

Retention of the three normal α-globin gene results in a silent carrier state and the $-\alpha^{3.7}$ deletion is the most common single gene disorder worldwide. The hematologic parameters of the individuals who have a single α-globin gene almost always normal and they are clinically well with no RBC alterations (Rappaport, V.J., 2004) (Table 1.2).

(b) α-thalassaemia trait : Loss of a two α-globin gene

The α-thalassaemia trait can be caused by heterozygous α-thalassaemia-1 (--/αα) or homozygous α-thalassaemia-2 (-α/-α). The most frequent cause of α-thalassaemia trait is homozygosity for the $-\alpha^{3.7}$ deletion ($-\alpha^{3.7}/-\alpha^{3.7}$). While the less frequent --/αα genotype can also underlie this phenotype, most commonly in Southeast Asian populations. This genotype is usually associated with a significant microcytosis and hypochromia, an elevated red cell count but normal or borderline low haemoglobin level. The haemoglobin electrophoresis is normal however MCV is decreased. (John, S.W., & David, H.K.C, 2001) (Table 1.2).

(c) Hb H disease

Haemoglobin H disease is an inherited haemoglobin disorder in which three of the four *α globin* gene are deleted or have mutation. There is a marked excess of β-globin chains, which are unstable, precipitate within the cell and lead to destruction of the red blood cells. Hb H disease is the most severe of the α-thalassaemia phenotypes compatible with life. It most frequently results from the interaction of α-thalassaemia-1 and α-thalassaemia-2 and it is commonly found in Southeast Asia and Mediterranean. Beside that Hb H disease may also result from the interaction of nondeletion mutations (Vefik, A., & Secil, G.A, 2012).

The clinical picture of Hb H disease is that of a chronic haemolytic anaemia of variable severity. Individuals with Hb H disease have moderate to severe anaemia but seldom requires transfusions except possibly during infection and during pregnancy. The degree of anaemia in Hb H patients with deletion of two α globin gene plus nondeletional α2 globin gene mutation is more severe than those with deletion of three α globin gene and in some rare cases Hb H disease can lead to hydrops foetalis with intrauterine demise. (John, S.W., & David, H.K.C, 2001).

(d) Hb Bart's Hydrop Foetalis

Hb Bart's hydrop foetalis or homozygous α^0-thalassaemia is the most severe form of α-thalassaemia, resulting from a complex lack of α-chain production, which always deletional in origin. If both parents are carrier of α^0-thalassaemia with deletion of both α-globin genes in cis, there is a 25% risk in each pregnancy that the foetus might have inherited both parental mutations. The affected foetus have inherited deletion completely remove the entire four α-globin gene. Many of these foetuses survive to the second or even third trimester of gestation or death within hours after death. These embryos also suffer from severe anaemia and hypoxia in utero. Apart from foetal death, Hb Bart's hydrop foetalis is also have risk of severe maternal complications that include pre-eclampsia, hypertension, toxaemia, antepartum haemorrhage, premature onset of labour and postpartum haemorrhage (Wee, Y.C *et al*, 2005) .

Table 1.2 : α-thalassaemia syndromes (Adapted from Waye and Chui, 2001)

Clinical Syndrome	**α-globin genotype**	**Clinical and laboratory findings**	**Reproductive significance***
Normal	αα/αα	Clinically Well	None
α-thalassaemia-silent carrier	-α/αα α^{Th}α/αα or ααTh/αα	Normal Hb level. Normal or borderline low MCV. Clinically well.	Hb H disease
α-thalassemia trait	-α/-α α^{Th}α/-α or ααTh/-α	Normal or borderline low Hb level. Low MCV.Clinically well	Hb H disease
	--/αα	Normal or borderline low Hb level. Low MCV.Clinically well	Hb H disease. Hb Bart's hydrops foetalis
Hb H disease	-α/-- α^{Th}α/-- or ααTh/--	Moderate anaemia. Low MCV. Usually not transfusion dependent.	Hb H disease. Hb Bart's hydrops foetalis
Hb Bart's hydrops foetalis	--/--	Severe anaemia and hypoxia in utero. Foetal death in 2nd and 3rd trimester or death within hours after death. Risk of severe maternal complications.	Not applicable.

*Potential risk of foetus inherited with these α-thalassaemia syndromes, depending on partner's α-globin genotype.
Hb=haemoglobin, α^{Th}=non deletional α-thalassaemia mutation involving either α2- or α1-globin gene, MCV=mean corpuscular volume.

1.2.6 Carrier screening of α-thalassaemia

1.2.6.1 Peripheral Blood Film

The first essential test that need to be performed was full blood cell count (FBC) looking for anaemia, microcytosis and hypochromia. The most important diagnostic criteria to detect thalassaemia carrier are microcytosis or hypochromia. Microcytosis refer to mean corpuscular volume (MCV) <80fl while hypochromia are mean corpuscular haemoglobin (MCH) <27pg (Leung, W.C *et al*, 2008). It is well known that the patients with α^0-thalassaemia are not anaemic but do have microcytosis. Therefore it is important physician pay attention not only to the Hb levels, but also the MCV and MCH levels (Chui, D.H., & Waye, J.S., 1998).

1.2.6.2 Hb H inclusion test

The Hb H inclusion test is used extensively in screening for subject with α-thalassaemia. The test based on incubation of erythrocyte with brilliant cresyl blue. It is quite laborious to scan for Hb H granules and faulty incubation of erythrocyte may give rise to false-positive results. The Hb H inclusion body test does not detect α^+-thalassaemia but is moderately sensitive and highly specific for α^0-thalassaemia trait due to the SEA, FIL, and MED deletions (Fucharoen, G., 2014).

1.2.6.3 Hb Analysis

(a) High Performance Liquid Chromatography (HPLC)

Haemoglobin (Hb) analysis and quantification of Hb A_2 should be performed as part of screening methods to diagnose thalassaemia carriers. Carriers of α-thalassaemia trait have Hb A_2 level <3.5% and for the elevated Hb A_2 level (>3.5%) the person maybe a carrier of β-thalassaemia (John, S.W., & David, H.K.C, 2001). DNA analysis for α-thalassaemia is necessary for diagnosis.

(b) Capillary Electrophoresis

Capillarys Electrophoresis (CE) is the recent most advanced technology which provides walkway convenience for electrophoresis. This CE approved method offers quantitation and detection of normal and abnormal haemoglobins, as an aid in the diagnosis of haemoglobinopathies and thalassaemias. With this technique, charged Hb molecules are separated by their electrophoretic mobilities in alkaline buffer with a fixed pH. Separation also occurs according to the electrolyte pH and electro osmotic flow created from negative charges on the capillary wall that shorten the analysis duration haemoglobinopathy and thalassaemia detection (Srivorakun, H., 2011).

(c) Gel Electrophoresis

Electrophoresis of haemoglobin on agar at acidic pH has been introduces some forty years ago.