REVIEW ARTICLE

Clinical and Haematological Parameters of Commonly Reported Non-deletional α-thalassaemia Mutations in Southeast Asia: A Review

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ABSTRACT

Alpha (α)-thalassaemia is a common genetic disorder worldwide caused by the deletion and rarely non-deletional mutations of the α-globin gene. Nearly 70 types of non-deletional mutations have been reported worldwide, and this review focuses on the common ones affecting α-thalassaemia patients. The common mutations are initiation codon mutation, codon 30, haemoglobin (Hb) Constant Spring, Hb Quang Sze, Hb Adana and Hb Evora. The haematological parameters of non-deletional mutations usually show mild changes. However, a severe reduction in haemoglobin level, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin count (MCHC) has been observed among compound heterozygous HbH disease, involving both deletional and non-deletional mutations. Although non-deletional mutations are rarely reported, it requires the study of more cases to understand the clinical phenotypes that lead to severe clinical manifestations.
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INTRODUCTION

Thalassaemia is a common genetic disorder that equally affects men and women (1). The estimated carrier rate of the disease is one to five percent of the world's population (2). Thalassaemia occurs due to a reduction or absence in the production of alpha- (α) or beta- (β) globin chains, which are required for the production of haemoglobin (Hb) A in red blood cells (RBC). This disrupts the functions of the RBC, leading to a lack of oxygen molecules being transported to organs and tissues. The classic symptom of thalassaemia patients is anaemia, which causes them to feel lethargic and have pale skin. Severe symptoms include bone problems, enlarged spleen and dark urine, besides stunted growth in children (3).

Cases of α-thalassaemia is reported at a higher frequency, affecting approximately five per cent of the global population (4), compared to 1.5 % for those with β-thalassaemia (5). The α-globin protein is expressed by genes known as haemoglobin subunit alpha 1 (HBA1) and haemoglobin subunit alpha 2 (HBA2). The normal individual carries four functional α-globin genes on chromosome 16, located in a 30 kb-region known as the α-globin locus. Therefore, mutations (deletion or non-deletion) in the genes will result in the absence or decrease in the production of α-globin chains (6). Figure 1 shows the α-globin genes cluster.

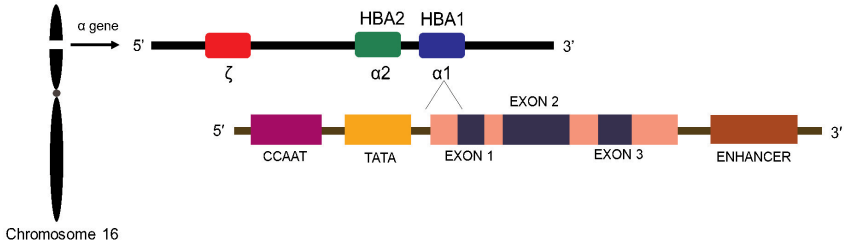

Figure 1: Schematic diagram of α globin genes located on chromosome 16

Deletional α-thalassaemia is more common than non-deletional disease (7). However, some non-deletional mutations may be prevalent in certain areas (8), such as Hb Constant Spring (CS), which is commonly reported in Southeast Asia, China and the Mediterranean. It is also the most dominant α-chain mutation variant in Southeast Asia (2).

NON-DELETIONAL α-THALASSAEMIA

Nearly 70 types of non-deletional α-thalassaemia have been reported worldwide, and most mutations occur in HBA2 rather than HBA1. In normal circumstances, the HBA2 is highly expressed compared to HBA1 in the ratio of three to one (8). Therefore, non-deletional mutations in HBA2 will have greater effect on the production of functional α-globin chains (9). Basic gene regulation in the production of globin chains involves transcription, translation, and post-translational modifications. The different types of mutations in HBA will cause abnormalities in gene expression. Abnormal gene expression will affect protein synthesis and structure of the α-globin chains (9). Thus, mutations will result in the formation of unstable Hb variants, which precipitates in RBC. The precipitation causes insoluble inclusion bodies (Heinz bodies) to form, which will eventually damage or destroy the RBC membrane, causing haemolysis and ineffective erythropoiesis (9). Highly unstable Hb variants due to point mutations in α-globin genes include Hb Evora, Hb Heraklion, Hb Dartmouth, Hb Quong Sze (QS), Hb Petah Tikva, Hb Aghia Sophia, Hb Suan Dok and Hb Adana. These variants have altered globin chain structures that affect the tetramer stability (9). Termination codon mutations will alter the stop codon at position 142 of HBA2 coding sequence, and such variants include Hb CS, Hb Icaria, Hb Seal Rock, Hb Pakse and Hb Koya Dora. These mutations will cause the production of elongated α-chains and highly unstable Hb variants expressed in a small amount (9).

In southeast Asia, the allele frequency of non-deletional α-thalassaemia in Thailand was recorded between 1.57 % and 1.67 %, followed by Cambodia at 8.0 % and Vietnam at 14.3 %. In Peninsular Malaysia, the distribution was quite high at 16.25 % (10). The commonly reported non-deletional α-thalassaemia mutations in Southeast Asia are initiation codon mutation (c.2delT), codon 30 (c.91_93delGAG), Hb CS (c.427T>C), Hb QS (c.377T>C), Hb Adana (c.179G>A) and Hb Evora (c.106T>C) (11, 12). Although non-deletional mutations are less common than deletional ones, they may show more severe clinical phenotypes because they are associated with protein stability (13).

Molecular characterisation of common non-deletional α-thalassaemia

A novel mutation identified at the initiation codon of HBA2 has led to the conversion of ATG to A-G. This mutation eliminates the NcoI restriction site, which may be identified by polymerase chain reaction and Southern blot analysis (14). It results in the reduction of mRNA expression and instability of transcribed mRNA, thus, being translated into unstable α-globin chains (15). The mutation in codon 30 of HBA2 results in codon deletion. The codon that encodes glutamic acid is deleted (delta (Δ)GAG), hence the α-globin proteins will be produced with 140 amino acids instead of 141. The deletion results in the Dde1 recognition site (16). This will lead to the production of an unstable α-globin chain, which eventually results in RBC destruction (17).

Hb CS is an abnormal Hb produced due to a mutation in the termination codon of HBA2 (18) and is the most common non-deletional mutation found in Southeast Asia. The mutation results in the insertion of glutamine in the stop codon that disrupts its function, leading to the production of elongated α-globin chains with additional 31 amino acid residues (2). Although Hb CS is prevalent in Southeast Asia and China, it is also reported in the Middle East and the Mediterranean. The allele frequency of Hb CS in Southeast Asia is observed at between 1 % and 8 % (19). In Malaysia, a previous study found the highest frequency among the Malay population at 2.24 %, followed by Chinese (0.66%) and Indians (0.16%) (20). In Indonesia, the figure was reported as 6 % of the overall population (21), followed by 1 % to 8 % in Thailand (22), 5 % to 6 % in Laos (23), 2 % in Cambodia (21) and 7 % in Myanmar (24). In Vietnam, there is a vast difference of frequency between populations in the country's south, where it is reported from 0 to 4 % (21), and among the Co-Tu people in the central zone, where it may be as high as 25 % (25). The frequency of Hb CS in Southeast Asian countries is shown in Figure 2.

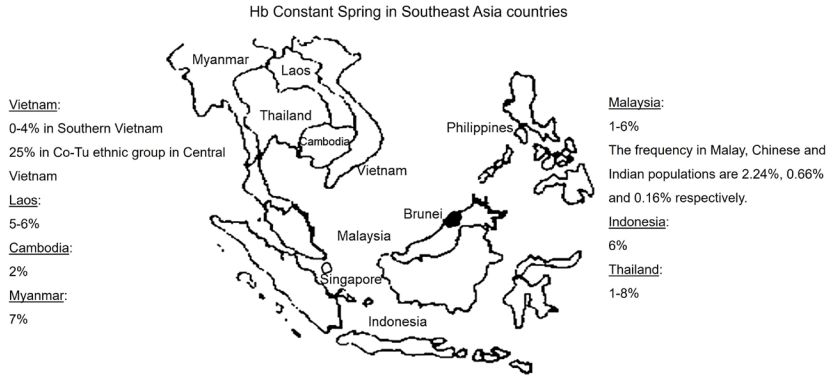

Fig. 2: Hb CS distribution frequency in Southeast Asia countries based on (2, 20-25, 29, 30, 34, 70, 71)

Meanwhile, codon 125 mutation in HBA2 results in Hb QS, which produces proline instead of leucine. The mutation abolishes the original EcoR1 restriction site and creates new sites for HpaII and MspI (26). The globin protein product consists of six α-helices, including A, B, E, F, G, and H (27), but the proline amino acid will disrupt the function of the H helix and cause a delay in the formation of α-β dimers, leading to the production of unstable Hb chains (26). Hb QS is of southern China origin, which has been carried down to Thailand through migration, resulting in hereditary mixing between Indochina populations. As a result, the mutation has become widely distributed throughout Thailand (28). This Hb variant is also more prevalent among Malaysian Chinese compared with Malays (29). Although it has been reported in Thailand and China (20), Hb QS is still rare, with less than 1 % only of the Chinese population in Malaysia bearing this mutation (30).

Hb Adana originates from Turkey, and is named after a city in the country's south where the variant was first discovered in 1993 (31). However, the number of cases reported there (0.5 % to 0.6 %) was relatively low compared to Indonesia (16 %) and Malaysia (the incidence ranges from 1.0 % to 21.4 % in several studies) (32-34). In addition, the incidence was higher in Sarawakian natives at 3.7 %, compared with Malay, Chinese and the Orang Asli at 1.7 %, 0.14 % and 1.9 %, respectively (29). Hb Adana occurs due to a point mutation in codon 59 of either HBA1 or HBA2. The mutation results in the substitution of small non-charged glycine to highly-charged aspartic acid coded by the codon. Initially, glycine is located at the internal position of the E helix, which is in contact with the glycine residue of the B helix. However, the aspartic acid substitute will cause steric hindrance, disrupting the production of a functional protein that causes an unstable α-globin chain to form (16, 34). A study suggested that Hb Adana in Malaysian populations may have been due to gene flow from Indonesia as a result of migration and mixed marriages (29). Singapore reported 3 % of its population as carriers of α-thalassaemia (35), and the common non-deletional mutations are Hb CS, Hb QS and Hb Adana (36). The frequency of certain mutations is not available as there is a lack of data on the distribution of non-deletional mutations across Southeast Asian countries.

The missense mutation of HBA2 in codon 35 results in the production of proline instead of serine, leading to the production of a variant known as Hb Evora. Proline is incapable of participating in the formation of α-helix proteins, which causes a conformational change in α-globin chains (37). This mutation was reported among patients of Caucasian descent in Portugal. The distribution of this mutation remains unknown as it is rarely reported (38). The first case related to this mutation was reported in 2001, with the patient showing mild microcytosis and was hypochromic (38).

Apart from the commonly identified variants, rare ones have also been reported. Hb Pakse, which is commonly found in central Thailand, is caused by a mutation at the termination codon of HBA2, which changes the original TAA, stop codon into TAT (which encodes tyrosine) (39). Mutation at the termination codon of HBA2 will lead to production of another elongated polypeptide variant known as Hb Kora Dora. However, this variant is found in a specific population, whereby a 10 % incidence was recorded in the Koya Dora tribes' people of Andhra Pradesh in India (8). The Hb variants identified might precipitate in the RBC membrane and could lead to haemolysis and ineffective erythropoiesis (2).

Hb Chesapeake is a variant caused by the presence of leucine instead of arginine as a result of a G to T substitution at codon 92 of the α-globin. It is observed to have a high affinity to oxygen (40), and the mutation affects the amino acids involved in the α1-β2 chain contact. It alters the normal rotational transition of Hb from a low-affinity to high-affinity oxygen state. Therefore, the Hb cannot release its oxygen molecules into tissues and organs, leading to tissue hypoxia. Hb Chesapeake has been reported among German, French and Irish families, and also in Japan (41).

Hb G Philadelphia is a normal and stable oxygen carrier due to a mutation in codon 16 of HBA1, in which lysine is substituted with glutamic acid (2). The prevalence of this Hb variant is estimated to be one in 5,000 African Americans. However, HbG Philadelphia is also reported in various ethnic groups, including Africans, Caucasians, Asians, Italians, Sardinians, and a few Chinese families (42).

CLINICAL SIGNIFICANCE OF NON-DELETIONAL α-THALASSAEMIA

α-thalassaemia is divided into thalassaemia trait, thalassaemia intermedia and thalassaemia major based on clinical severity. Non-deletional type carriers may experience variable clinical manifestations, from being asymptomatic to having mild symptoms (7). The clinical severity depends on the mutation, whether it fully or partially reduces the production of remaining α-globin chains (43). Usually, non-deletional mutations will lead to Hb variants without any serious clinical significance; hence, if the mutations occurred in an important amino acid residue, it would cause a reduction in the production of α-globin chains that results in haemolytic anaemia (30).

However, the presence of compound heterozygous Hb variant of non-deletional and other types of α-thalassaemia mutation is more unstable and produces severe clinical presentations, such as haemoglobin H (HbH) disease (30). Non-functionality of HBA2 resulting from deletions will be compensated with increased expression of HBA1. As a result, the interaction between

non-deletional and deletional mutations will lead to severe phenotypes than deletional mutations alone. Patients with compound heterozygous HbH disease with non-deletional defects (--/αNondeletionalα) have more severe clinical presentations, such as anaemia, jaundice, hepatosplenomegaly, bone changes and frequent requirement of blood transfusion (7). Excess β-globin chains will precipitate to form β4, known as HbH, which also causes RBC haemolysis due to cell membrane damage (44). However, in some compound heterozygous cases, it involves one severe non-deletional mutation and a non-expressing allele due to deletion. This results in a condition that lies between HbH disease, Hb Barts and hydrops foetalis (9). The absence of α-globin chain production will lead to Hb Barts, where the Hb is made of four α-globin chains (45). This is a serious condition affecting foetuses, which will suffer intra-uterine anaemia, brain oedema and growth retardation, with high risk of intrauterine or post-natal fatality (9).

HAEMATOLOGICAL CHANGES IN NON-DELETIONAL MUTATIONS

The haematological parameters varied among α-thalassaemia patients, depending on the number of α-genes altered by mutation. Generally, haematological parameters used to identify α-thalassaemia are based on a full blood count (FBC) that includes Hb level, RBC count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and reticulocyte count (46). In general, the level of Hb in α-thalassaemia varies from normal to severe anaemia (Hb 7.5-15.5 g/dl). Meanwhile, there is a reduction of MCV (<79 fL), MCH (<27 pg) and normal-to-slight decrease in HbA2 level, depending on the number of functional α-globin genes (9). Heterozygous α+-thalassaemia (-α/αα) will have normal red cell indices or a slight reduction in Hb, MCV and MCH values (21). Patients with less than 25 pg of MCH indicates either an α0-thalassaemia trait or homozygosity for α+-thalassaemia trait, rather than α+-thalassaemia heterozygosity (21). However, the variations in MCV and MCH values are linked to the underlying mutation in the α-globin genes (46). The determination of MCH is more sensitive than MCV because RBCs stored at room temperature tend to swell over time, hence decreasing MCV sensitivity. Therefore, MCH is more useful in aiding the diagnosis of thalassaemia (47).

The standard laboratory technique to detect thalassaemia involves the quantification of HbA2 and Hb F using high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). The percentage of HbA2 (2.5 to 3.5 %) and Hb F (less than 1 %) (48) in α-thalassaemia carriers are within the normal range, whereas a reduction in HbA2 (1.5 % to 2.5 %) is observed in α-thalassaemia trait patients with two affected genes (49, 50). The laboratory diagnosis for HbH and Hb Barts may also be detected by HPLC and CE. HbH and Hb Barts are indicated by the fast eluting HPLC peaks (51), besides a sharp peak in the first minute of elution (52).

INITIATION CODON MUTATION

The haematological phenotype of individuals with a mutation in the initiation codon of HBA2 will show a decrease in MCV and MCH levels, while a normal level of HbA2 is observed (15). Hypochromic microcytosis may be seen in the blood-film slides. Specifically, this mutation will reduce the α-globin gene transcription by 50 %. The mRNA transcribed from the mutated gene will produce non-functional α-globin chains during translation (15). Therefore, this condition will result in haemolytic anaemia. Furthermore, patients with HbH disease caused by mutation of initiation will suffer from anaemia, decreased HbA2 level, and a slight rise in Hb F level. However, this condition does not require a blood transfusion, and it does not affect the growth and development of the children (53).

CODON 30 MUTATION

Mutation at codon 30 of HBA2 will result in microcytosis, and a decrease in MCV and MCH levels. Furthermore, this mutation may not only cause a reduction in α-globin chain production as it degrades rapidly after translation, but also affects the RBC production and leads to ineffective erythropoiesis (17, 54). This mutation produces a highly unstable protein that tends to degrade rapidly after translation, thus reducing the level of functional α-globin chains. Consequently, the unstable variant is not detectable using CE (54). In addition, the mutation will also cause proteolytic destruction in RBC due to the unstable α-globin chains. This condition may affect the proteolytic activity of erythroid precursors and lead to ineffective removal of excess α-chains in the foetus (17). Individuals with a point mutation in codon 30 may present with a similar clinical phenotype as Hb QS patients (54).

HAEMOGLOBIN CONSTANT SPRING

In non-deletional α-thalassaemia, the laboratory diagnosis will show mild changes in RBC indices. However, when point mutations occur in the region that is mainly involved in the expression of α-globin chains, it will show a variation in laboratory parameters. Hb CS is the most unstable variant as the mutation results in overhydrated RBC that are prone to haemolysis (55). Heterozygous Hb CS patients tend to suffer from lower haemoglobin level than in usual heterozygous thalassaemia individuals (21). The level of Hb CS in the heterozygous patient is from 1 % to 2 %, while it comprises 5 % to 6 % in homozygous patients. Most patients, either homozygous or heterozygous, are asymptomatic with normal haematological parameters,

including the MCV level, though some homozygous patients may present with mild anaemia and reduced MCV (25).

Since the MCV and MCH levels in these patients are variable (2), detection of Hb CS may be missed if the MCV cut-off level of <80 fL is used (47). The detection of Hb CS in HPLC is indicated by a peak in window C, but in some heterozygous cases with low-level Hb CS, the diagnosis is often missed. However, low-level Hb CS is detectable in CE, which will show a peak in the HbC/CS zone. Therefore, the CE method is superior to HPLC in detecting Hb CS, especially in the heterozygous state (56). The Hb F level in Hb CS patients is within normal range, and there is a slight reduction in HbA2 level. Usually, the level of Hb CS is between 2 % and 11 %, and in some conditions, it may be lower or undetectable (21). Figure 3 shows the positions of HbA2, Hb F, HbA, and Hb CS with the retention time in a HPLC result.

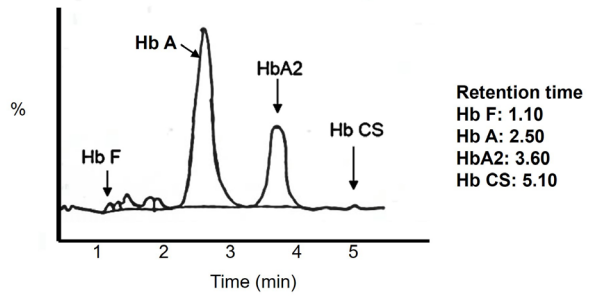

Figure 3: Position of HbA2, Hb F, Hb A and Hb CS with the retention time respectively in the HPLC system

Homozygous Hb CS patients will usually present with mild anaemia. However, the clinical outcome varies, depending on age. A mild to severe anaemia may occur in children and adults, whereas neonatal jaundice can present in neonates (18). However, there was a reported case involving a neonate who was initially diagnosed with foetal anaemia, and later, developed signs of hydrops foetalis due to homozygous Hb CS (57). Severe jaundice requiring blood transfusion has been observed in foetuses and neonates with homozygous Hb CS (18).

In some cases, Hb Pakse has been misdiagnosed as Hb CS because the Hb could not be differentiated in HPLC and CE (21) as it migrates in the same window and band. Both mutations occur in the termination codon, resulting in extended α-globin chains. In addition, there are no significant differences in the haematological parameters between the mutations. However, the HbH level is higher in Hb Pakse patients. So, the phenotype of these mutations is similar, which may lead to misdiagnosis (58). However, multiplex allele-specific polymerase chain reaction or genomic sequencing can distinguish between the mutations (21). Therefore, electrophoresis is not sufficient for an accurate diagnosis for such Hb disorder, and DNA analysis is needed for a definitive diagnosis.

HAEMOGLOBIN QUONG SZE

Hb QS is detectable when using a MCH cut-off value of <27 pg than detecting the Hb variants with an MCV cut-off of <80 fL (47). So, individuals with a mutation in codon 125 would have a MCH value of <27 pg and may have a low to normal level of MCV. Apart from that, these patients will experience mild to moderate anaemia with haemolysis and ineffective erythropoiesis (2). Homozygous Hb QS is rarely reported because it is less prevalent in the gene frequency. However, homozygous Hb QS will result in HbH disease, with the presence of Hb Barts in the range of 10 % to 30 % in the blood. Therefore, the foetus would have severe anaemia and in a blood smear, its RBC will be seen as microcytic and hypochromic, with mild anisopoikilocytosis, basophilic stippling, and a marked increase in nucleation. Babies born with this condition would also require regular blood transfusions (59). However, the Hb QS is a highly unstable Hb, so it is undetectable in routine electrophoresis and HPLC methods (30).

HAEMOGLOBIN ADANA

Hb Adana is formed due to unstable molecules and there are no significant haematological parameters to confirm the diagnosis (60). Hb Adana carriers are asymptomatic and may experience mild anaemia only (61), and their levels of MCV and MCH are variable (2). The RBC indices are similar to those with α-thalassaemia traits due to gene deletions (61). Generally, the haematological parameters are within the normal range. The HPLC method for Hb subtypes is unable to detect Hb Adana, while the subtyping and routine haematology testing for heterozygous Adana were normal, therefore requiring molecular detection for confirmatory diagnosis (62).

Patients with homozygous Hb Adana (αcodon 59α/αcodon 59α) are often associated with hydrops foetalis syndrome even though they still have two more functional α-globin genes, whereby the foetuses with homozygous genes normally die at 22 to 25 weeks of gestation. At the molecular level, an amino acid substitution occurs in the HBA that compromise the α-globin chain stability, leading to precipitation and damage to the RBC membrane, and causing haemolysis. This occurs especially when the dominantly expressed HBA2 mutation leads to hydrops foetalis or HbH disease even in the homozygous condition (63). However, compound heterozygous patients with Hb Adana on the HBA1 with α0-thalassaemia will not show features of hydrops foetalis, but severe anaemia has been reported. This may be caused by the higher rate of α-globin chain transcription occurring in HBA2 than in HBA1 (64). Furthermore, the severe phenotypes observed in homozygous patients may not be due to a decrease in the α-globin chain synthesis, but due to the formation of

α-globin chain variants interfering with normal tetramer formation or damaging the RBC (65). The unstable Hb variant will result in haemolytic anaemia.

HAEMOGLOBIN EVORA

Patients with Hb Evora have mild erythrocytosis, microcytosis, hypochromic anaemia and normal HbA2 level (38). The MCH and MCV levels among patients were variable as the usual cut-off point for the parameters could not be used to rule out the mutation. The Hb level ranges from 12 g/dl to16 g/dl (66). In Hb Evora patients, the level of HbA2 is normal, and the variant is undetectable by HPLC method (38). Carriers of this mutation usually have abnormalities in haematological parameters, but they have a normal mRNA level as the mutated globin chains produced are terminated by proteolysis (66). Therefore, in this case, molecular diagnosis is essential to identify the mutation.

COMPOUND HETEROZYGOSITY OF α-THALASSAEMIA

The majority of HbH diseases reported in Southeast Asia were caused by gene deletions. However, 20 % to 40 % of the cases are caused by compound heterozygosity of deletional and non-deletional mutations (30). A few examples of compound heterozygous cases that have been reported are HbH disease with Hb CS, followed by HbH with Hb QS (44). A case was reported involving Hb QS, which showed an asymptomatic, unstable variant with mild microcytic anaemia. However, in the case of Hb QS compound heterozygosity with two Southeast Asia (SEA) deletions of α-globin genes resulted in HbH disease with severe haemolytic anaemia (30). Compound heterozygosity for α0 and non-deletional α-globin gene resulted in severe HbH disease, such as the following genotype ––SEA/αCSα, ––SEA/αPakseα, –SEA/αQSα or αCodon 59α/ ––SEA. In these cases, the clinical outcome was severe and resulted in splenectomy in most non-deletional cases. Individuals with HbH-CS have a higher value of MCH and MCV (18.6 pg, 65.2 fl) compare to HbH group (16.6 pg, 54.0 fl) (67). A lower level of Hb with high bilirubin and reticulocyte count poses a greater risk of severe anaemia, thus requiring blood transfusions (21).

Most HbH disease among deletional α-thalassaemia patients does not require a blood transfusion. However, it is mostly required in both deletional and non-deletional diseases (21). Furthermore, gene abnormality due to non-deletional α-thalassaemia leading to HbH disease will result in anaemia, reticulocytosis and hypochromia. On the other hand, a decrease in MCV, MCH, Hb, and MCHC levels in cases of Hb CS and deletional mutations was detected. These results are due to imbalance production of α and β globin chains. Thus, greater Hb CS levels were seen in HPLC for those cases (55). A reduction in MCHC level (68) may be due to the overhydration of cells, which is specifically identified among patients with the Hb CS mutation (69). Apart from that, the mean value of MCV, MCH and MCHC in non-deletional cases are 76 fL, 22.1 pg and 29.2 fL, while for deletional cases, they are 64 fL, 20 pg and 31.3 fL (69). The Hb level in non-deletional HbH disease is less than 7g/dl, hence, it may have significant hepatosplenomegaly, growth retardation and requirement for frequent blood transfusion (8). Hydrops foetalis is a result of mutation in all α-globin genes, and it is the most severe form of α-thalassaemia as no α-globin chain will be produced. It is a common disease among deletional α-thalassaemia; however, it is rarely reported as a compound heterozygosity of α0 and non-deletional α-thalassaemia (16). Table I shows the summary of Hb variants that were discussed. The minor alteration in the haematological parameters of the non-deletional carrier may lead to misdiagnosis. Therefore, molecular analysis is required for decisive diagnosis of the disease.

Table I: Summary of common non-deletional α-thalassemia mutations reported in Southeast Asia

Mutated point in the α genes	Affected gene	Designated name	Amino acid sequence	References
Initiation codon	HBA2	-	ATG A-G	(14)
Codon 30	HBA2	-	ΔGAG	(16), (53)
Codon 35	HBA2	Hb Evora	TCC CCC	(37), (65)
Codon 59	HBA1 or HBA2	Hb Adana	GGC GAC	(33), (2)
Codon 125	HBA2	Hb Quang Sze	CTG CCG	(28), (2)
Termination Codon	HBA2	Hb Constant spring	TAA CAA	(17), (20)

CONCLUSION

Alpha-thalassaemia is a common genetic disorder worldwide, thus, requires proper care and management. Although deletional α-thalassaemia is common than non-deletional ones, the latter will result in a more severe and significant clinical presentation. The discussed haematological parameters of non-deletional genes showed that MCH and MCV levels play a critical role in identifying patients with the disorder. However, the RBC indices solely are not an effective screening tool for thalassaemia. Further studies on the rare mutations in HBA genes will increase the knowledge in understanding the unexplained clinical phenotypes related to α-globin gene regulation. Moreover, this will prove beneficial, efficient and provide better management of the disease, besides encouraging research to find an effective cure.

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