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ORIGINAL ARTICLE

Molecular characteristic of alpha thalassaemia among patients diagnosed in UKM Medical Centre

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Abstract

Alpha (α) thalassaemia is the most common inherited disorder in Malaysia. The clinical severity is dependant on the number of α genes involved. Full blood count (FBC) and haemoglobin (Hb) analysis using either gel electrophoresis, high performance liquid chromatography (HPLC) or capillary zone electrophoresis (CE) are unable to detect definitively alpha thalassaemia carriers. Definitive diagnosis of α-thalassaemias requires molecular analysis and methods of detecting both common deletional and non-deletional molecular abnormailities are easily performed in any laboratory involved in molecular diagnostics. We carried out a retrospective analysis of 1623 cases referred to our laboratory in Universiti Kebangsaan Malaysia Medical Centre (UKMMC) for the diagnosis of α-thalassaemia during the period October 2001 to December 2012. We examined the frequency of different types of alpha gene abnormalities and their haematologic features. Molecular diagnosis was made using a combination of multiplex polymerase reaction (PCR) and real time PCR to detect deletional and non-deletional alpha genes relevant to southeast Asian population. Genetic analysis confirmed the diagnosis of α-thalassaemias in 736 cases. Majority of the cases were Chinese (53.1%) followed by Malays (44.2%), and Indians (2.7%). The most common gene abnormality was $\alpha\alpha/--^{SEA}$ (64.0%) followed by $\alpha\alpha/-\alpha^{3.7}$ (19.8%), $-\alpha^{3.7}/--^{SEA}$ (6.9%), $\alpha\alpha/\alpha\alpha^{CS}$ (3.0%), $--^{SEA}/--^{SEA}$ (1.2%), $-\alpha^{3.7}/-\alpha^{3.7}$ (1.1%), $\alpha\alpha/-\alpha^{4.2}$ (0.7%), $-\alpha^{4.2}/--^{SEA}$ (0.7%), $-\alpha^{3.7}/-\alpha^{4.2}$ (0.5%), $\alpha\alpha^{CS}/--^{SEA}$ (0.4%), $\alpha\alpha^{CS}/\alpha\alpha^{Cd59}$ (0.4%), $\alpha\alpha^{CS}/\alpha\alpha^{CS}$ (0.4%), $-\alpha^{3.7}/\alpha\alpha^{Cd59}$ (0.3%), $\alpha\alpha/\alpha\alpha^{Cd59}$ (0.1%), $\alpha\alpha^{Cd59}/\alpha\alpha^{IVS\ I-1}$ (0.1%), $-\alpha^{3.7}/\alpha\alpha^{CS}$ (0.1%) and $--^{SEA}/\alpha\alpha^{Cd59}$ (0.1%). This data indicates that the molecular abnormalities of α-thalassaemia in the Malaysian population is heterogenous. Although α-gene deletion is the most common cause, non-deletional α-gene abnormalities are not uncommon and at least 3 different mutations exist. Establishment of rapid and easy molecular techniques is important for definitive diagnosis of alpha thalassaemia, an important prerequisite for genetic counselling to prevent its deleterious complications.

Keywords: Deletional α-thalassaemia, α-thalassaemia variant, molecular analysis, genetic polymorphism, Malaysian population

INTRODUCTION

Alpha (α) thalassaemia, is the most common inherited disorder of haemoglobin (Hb) synthesis worldwide and is commonly found in Southeast Asian, Mediterranean and Middle East populations. α-thalassaemia is a public health problem because when compared to beta (β) thalassaemia, the carrier status for α-thalassaemia cannot be detected by the usual screening methods using gel electrophoresis, high performance liquid chromatograpy (HPLC) or capillary zone electrophoresis (CE). In Malaysia, the prevalence of α-thalassaemia trait was higher compared to β-thalassaemia carrier, which is 15.8% versus 4.5%.[1]

The α-thalassaemia syndromes are characterized by reduced or absent α-globin synthesis

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resulting in globin chain imbalance. The relative excess of γ or β chains form the tetramers, Hb Bart's or Hb H, respectively leading to haemolysis. Normal persons have four α-globin genes. The clinical severity of α-thalassaemia is dependent on the number of α-genes affected by genetic mutations. Individuals with one or two α-gene deletions or mutations are usually asymptomatic. However, those with three α gene deletions have problems of thalassaemia intermedia and those with four α gene deletions are not compatible with life. The most common molecular abnormalities in α-thalassaemia are gene deletions. In a minority of cases non-deletional α gene abnormalities exist. In Malaysia, Hb Constant Spring (Hb CS) is the most common α-globin structural variant,[2] especially among Malay races,[3] and recently, a few cases of Hb Adana have been detected.[4-6] Hb CS involves a TAA → CAA base pair substitution in the termination codon of the α2-globin gene. The end product is an elongated α-globin chain with additional 31 amino acid residues. Hb Adana is due to GGC→GAC base pair substitution in codon 59 of the α1 or α2- globin gene.

Most cases of α-thalassaemia will show some red cell indices abnormalities. Some cases may show almost normal Hb level especially those with single gene deletion or mutation with minimal abnormalities in other haematology parameters. The British Committee for standards in Haematology recommended MCH value of less than 27 pg as the parameter for primary screening to quantify Hb subtypes.[7]

Even though Hb analysis using either gel electrophoresis, HPLC or CE is unable to detect α-thalassaemia trait, the tests are useful for the diagnosis of the more severe forms which are Hb H disease (3 α genes deleted, genotype α-/--) and Hb Bart's hydrops fetalis (4 α genes deleted, genotype --/--). Hb electrophoresis will show the fast H and Barts band. Analysis using either HPLC or CE is able to detect the Hb H and Hb Barts. As for non-deletional α-globin variant cases such as heterozygous Hb CS, the gel electrophoresis and HPLC method always miss to identify these cases due to instability of its mRNA.[8] CE method showed a better detection of heterozygous Hb CS cases.[9]

Molecular analysis is a definitive method for diagnosis of α-thalassaemia carriers. The molecular techniques used for the identification of deletion/mutations in the α-globin gene such as multiplex GAP polymerase chain reaction (PCR) for gene deletion and multiplex PCR for non-gene deletion, and real time PCR using allele-specific fluorescence for Hb CS, has now replaced the cumbersome and time-consuming Southern Blot analysis. Molecular characterization of α-thalassaemia in any given population is a prerequisite for the establishment of prenatal diagnosis and genetic counselling services.

The objectives of this study were to determine the prevalence of the different types of gene abnormalities and their haematologic features in alpha thalassaemias diagnosed in UKM Medical Centre.

MATERIALS AND METHODS

From October 2001 to Disember 2012, a total of 1632 blood samples were analysed by our Molecular Genetic Unit, Department of Diagnostic Laboratory Services, UKM Medical Centre. The samples were obtained from peripheral blood EDTA of patients sent to this laboratory for diagnosis of alpha thalassaemia. Informed consent were taken prior to the blood taking. The haemoglobin (Hb) concentrations and red cell indices were determined using an automated blood cell counter (Coulter LH 750^{R}). The quantitation of Hb A_2, Hb E, Hb A and Hb F were performed using automated high performanced liquid chromatography (HPLC) system, beta thalassaemia short (BTS) program (Bio-Rad VARIANT) and capillary zone electrophoresis (CE) (Sebia, Inc., Norcross, Ga). The latter method was introduced to our laboratory in 2009.

Genomic DNA was extracted from peripheral blood leucocytes using QIAamp DNA Blood Mini Kit (GENE ALL). Multiplex gap-polymerase chain reaction (PCR) amplification for detection of common α-thalassaemia gene deletion ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$) was performed on all cases, method as described by Samuel *et al*, 2000.[10] The PCR was carried out on the 9700 PCR Thermal Cycler (Applied Biosystem, USA). The amplified products were electrophoresed in agarose gel with Ethidium Bromide. DNA bands were visualized under ultra-violet light illumination. Analysis for α-thalassaemia gene deletions $--^{FIL}$, $--^{MED}$, $=\!=^{THAI}$ and $--^{20.5}$ types were commenced in 2012 to accommodate for the population demographic changes as a result of influx of immigrants.

Real time PCR using Taqman$^{@}$ SNP genotyping assays for detection of Hb CS was performed on cases that were negative for

α-thalassaemia gene deletion with thalassaemic red cell indices and cases that showed positive for Hb CS by HPLC or CE. This test was introduced in year 2006, using specific primers and probes for the point mutation. The probes were dual labeled TaqMan probes with both a fluorophore and a quencher dye (Biosearch Technologies), to detect amplification of specific DNA targets, method used as described by Vivian *et al*, 2001.[11] The real-time PCR was carried out using RotorGene 6000 (Corbett Research, Aus).

Multiplex-PCR amplification for non-deletional α-thalassaemia was started in our laboratory in 2012, method used as described by Eng *et al*, 2001.[12] The non deletional mutations screened were codon 59 GGC→GAC mutation or Hb Adana ($\alpha\alpha^{CD59}$), codon 125 CTG→CCG or Hb Quong Sze ($\alpha\alpha^{125}$), codon 35 TCC→CCC mutation ($\alpha\alpha^{35}$), codon 30ΔGAG mutation ($\alpha\alpha^{30}$), initiation codon ATG→A-G mutation ($\alpha\alpha^{\text{Initiation codon}}$) and termination codon TAA→CAA or Hb CS ($\alpha\alpha^{CS}$). However, this method of analysis was not discriminative for heterozygous and homozygous states. After we started the non-deletional α-thalassaemia services, we retrospectively evaluated the archival samples that showed thalassaemic indices but was negative with deletional α-thalassaemia PCR analysis.

RESULTS

From October 2001 to Dec 2012, 736 of 1623 cases were diagnosed to have deletional and/or non-deletional α-thalassaemia (826 alleles). Male to female ratio was 1:2. Majority of the cases were Chinese (53.1%) followed by Malays (44.2%), and Indian (2.7%).

The most common gene abnormality was $\alpha\alpha/--^{SEA}$ (64.0%) followed by $\alpha\alpha/-\alpha^{3.7}$ (19.8%), $-\alpha^{3.7}/--^{SEA}$ (6.9%), $\alpha\alpha/\alpha\alpha^{CS}$ (3.0%), $--^{SEA}/--^{SEA}$ (1.2%), $-\alpha^{3.7}/-\alpha^{3.7}$ (1.1%), $\alpha\alpha/-\alpha^{4.2}$ (0.7%), $-\alpha^{4.2}/--^{SEA}$ (0.7%), $-\alpha^{3.7}/-\alpha^{4.2}$ (0.5%), $\alpha\alpha^{CS}/--^{SEA}$ (0.4%), $\alpha\alpha^{CS}/\alpha\alpha^{Cd59}$ (0.4%), $\alpha\alpha^{CS}/\alpha\alpha^{CS}$ (0.4%), $-\alpha^{3.7}/\alpha\alpha^{Cd59}$ (0.3%), $\alpha\alpha/\alpha\alpha^{Cd59}$ (0.1%), $\alpha\alpha^{Cd59}/\alpha\alpha^{IVS\ I-1}$ (0.1%), $-\alpha^{3.7}/\alpha\alpha^{CS}$ (0.1%) and $--^{SEA}/\alpha\alpha^{Cd59}$ (0.1%) (Table 1).

Out of 736 cases, 657 (89.2%) cases were confirmed by genotyping as α-thalassaemia trait. There were 69 (9.4%) cases of thalassaemia intermedia (clinically symptomatic anaemia except for one case due to concomitant delta beta thalassaemia), majority showed three gene deletions and the other showed a combination of non-deletional and deletional gene abnormalities (Table 1). 10 cases (1.4%) were confirmed with alpha gene abnormalities consistent with hydrops foetalis (severe anaemia that was incompatible with life).

TABLE 1: Incidence of α thalassaemia in UKM Medical Centre

Genotype	Malay	Chinese	Indian	Overall
$\alpha\alpha/--^{SEA}$	144 (30.6%)	326 (69.2%)	1 (0.2%)	471 (64.0%)
$\alpha\alpha/-\alpha^{3.7}$	109 (74.7%)	19 (13%)	18 (12.3%)	146 (19.8%)
$-\alpha^{3.7}/--^{SEA}$	23 (45.1%)	28 (54.9%)	-	51 (6.9%)
$\alpha\alpha/\alpha\alpha^{CS}$	20 (90.9%)	2 (9.1%)	-	22 (3.0%)
$--^{SEA}/--^{SEA}$	1 (11.1%)	8 (88.9%)	-	9 (1.2%)
$-\alpha^{3.7}/-\alpha^{3.7}$	6 (75%)	1 (12.5%)	1 (12.5%)	8 (1.1%)
$\alpha\alpha/-\alpha^{4.2}$	5 (100%)	-	-	5 (0.7%)
$-\alpha^{4.2}/--^{SEA}$	1 (20%)	4 (80%)	-	5 (0.7%)
$-\alpha^{3.7}/-\alpha^{4.2}$	4 (100%)	-	-	4 (0.5%)
$\alpha\alpha^{CS}/--^{SEA}$	1 (33.3%)	2 (66.7%)	-	3 (0.4%)
$\alpha\alpha^{CS}/\alpha\alpha^{CD59}$	3 (100%)	-	-	3 (0.4%)
$\alpha\alpha^{CS}/\alpha\alpha^{CS}$	3 (100%)	-	-	3 (0.4%)
$-\alpha^{3.7}/\alpha\alpha^{CD59}$	2 (100%)	-	-	2 (0.3%)
$\alpha\alpha/\alpha\alpha^{CD59}$	1 (100%)	-	-	1 (0.1%)
$*\alpha\alpha^{CD59}/\alpha\alpha^{IVS\ I-1}$	1 (100%)	-	-	1 (0.1%)
$-\alpha^{3.7}/\alpha\alpha^{CS}$	-	1 (100%)	-	1 (0.1%)
$--^{SEA}/\alpha\alpha^{CD59}$	1 (100%)	-	-	1 (0.1%)
Total	325 (44.2%)	391 (53.1%)	20 (2.7%)	736

*Sample was sent for DNA sequencing

TABLE 2: Red cell indices in α-thalassaemia cases (means ± SD)

Types of thalassaemia	Types of α gene defect	No. of patients	Mean Hb (g/dl)	Mean RBC (10 x 10^9/L)	Mean MCV (Fl)	Mean MCH (pg)
Thalassaemia trait	αα/-$\alpha^{3.7}$	120	12.3 ± 1.5	5.0 ± 0.6	75.2 ± 7.8	24.7 ± 2.7
	αα/-$\alpha^{4.2}$	2	12.1 ± 2.4	4.9 ± 0.7	74.4 ± 5.9	25.6 ± 3.9
	αα/αα^{CS}	15	12.1 ± 1.7	4.7 ± 0.6	78.1 ± 7.1	25.8 ± 2.3
	αα/αα^{CD59}	1	12.30	5.21	74.30	23.60
	αα/--^{SEA}	379	11.8 ± 1.4	5.5 ± 0.7	67.0 ± 4.8	21.6 ± 1.7
	-$\alpha^{3.7}$/-$\alpha^{3.7}$	8	11.8 ± 2.1	5.1 ± 0.9	71.8 ± 7.8	23.4 ± 2.9
	-$\alpha^{3.7}$/-$\alpha^{4.2}$	4	11.3 ± 1.4	5.5 ± 0.4	64.0 ± 7.7	20.6 ± 3.0
Thalassaemia intermedia	-$\alpha^{3.7}$/αα^{CS}	1	12.6	5.75	65.9	22.0
	-$\alpha^{3.7}$/--^{SEA}	51	8.8± 1.1	4.8 ± 0.8	59.5 ± 6.3	18.6 ± 1.8
	-$\alpha^{4.2}$/--^{SEA}	5	9.9 ± 1.2	5.14 ± 0.9	63.5 ± 4.7	19.6 ± 1.6
	αα^{CS}/--^{SEA}	3	7.1 ± 1.4	3.6 ± 0.5	66.8 ± 7.7	19.8 ± 2.7
	-$\alpha^{3.7}$/αα^{CD59}	2	10.1 ± 1.3	4.1 ± 0.5	75.7 ± 0.4	24.3 ± 0.4
	αα^{CS}/αα^{CD59}	3	7.9 ± 1.0	3.2 ± 0.4	81.9 ± 3.9	24.8 ± 2.1
	αα^{CS} /αα^{CS}	3	9.7 ± 0.4	4.1 ± 0.3	77.9 ± 1.2	24.0 ± 0.9
	αα^{CD59}/αα$^{IVS\ I-1}$	1	5.80	2.78	65.9	20.9

Haematological parameters were analyzed in relation to the genotype (Table 2). Cases known to have underlying iron deficiency, chronic medical illness, pregnant women or concomittant with other thalassaemia or haemoglobinopathies were excluded from this analysis.

DISCUSSION AND CONCLUSION

In UKMMC, most cases sent to the laboratory are from clinics or wards for cases of incidental findings of abnormal red cell parameters, individuals with a family history of thalassaemia, symptomatic hypochromic microcytic anaemia and for prenatal diagnosis of hydrops foetalis for couples who were diagnosed with α^0-thalassaemia carriers.

The request for molecular analysis were made after Hb analysis showed normal findings in patients with hypochromic microcytic red cell indices or any known cases already diagnosed by Hb analysis but needed confirmation by molecular analysis.

Since the services started, 736 out of the 1623 patients were confirmed to have α-thalassaemia. The ethnic profile of the blood samples received showed the highest proportion in the Malay ethnic group (53.3%, 865/1623) followed by Chinese (42.1%, 683/1623), Indian (2.96%, 48/1623) and other ethnic groups (1.66%, 27/1623). This reflects the ethnic mixture of the local area served by the hospital. However, we found that the prevalence of α-thalassaemia is highest among the Chinese (53.1%, 391/736) followed by the Malays (44.2%, 325/736) and the Indians showing a very low prevalence (2.7%, 20/736). This was an unexpected finding since studies done by Wee *et al*, 2005[1] and Rahimah *et al*, 2012[13] showed that highest prevalence of α-thalassaemia occur among Malay ethnic group. We noticed that quite a number of requests from Chinese patients came as family screening with a background history of thalassaemia, while requests from Malay patients came as individuals.

In our analysis, 657 out of 736 cases showed genotype consistent with α-thalassaemia trait. These cases were heterozygous for an allele of either a single or two α-gene deletions, or α-gene mutations. A few of them were homozygous for a single gene deletion. Majority were heterozygous for the two gene deletion (αα/--^{SEA}) (71.7%, 471/657) consistent with α^0-thalaessaemia traits, followed by heterozygosity for the 3.7 kb single gene deletion (αα/-$\alpha^{3.7}$) (22.2%, 146/657) of α^+-thalaessaemia trait. The remaining 6.1% showed findings consistent with α^+-thalaessaemia traits, heterozygous for either 4.2 kb single gene deletion (αα/-$\alpha^{4.2}$) (0.7%), Hb Constant Spring single gene mutation (αα/αα^{CS}) (3.3%), Hb Adana single gene mutation (αα/αα^{Cd59}) (1.5%) or homozygous for 3.7 kb deletion (-$\alpha^{3.7}$/-$\alpha^{3.7}$) (1.2%) and compound heterozygous for 3.7 kb and 4.2 kb deletion (-$\alpha^{3.7}$/-$\alpha^{4.2}$) (0.6%). Majority of the α^o-thalaessaemia traits αα/--^{SEA}

(69.2%, 326 cases) were Chinese, while the α^+-thalaessaemia trait $\alpha\alpha/-\alpha^{3.7}$ most commonly found in the Malays (74.7%). These findings were consistent with results of study done by Wee *et al*, 2005.[1] In their study 15/25 (60%) Chinese patients had α^o-thalaessaemia of $\alpha\alpha/--^{SEA}$ genotype, while 43/64 (67.2%) Malay patients were α^+-thalaessaemia with $\alpha\alpha/-\alpha^{3.7}$. All Indians in this study showed α^+-thalaessaemia of $\alpha\alpha/-\alpha^{3.7}$ genotype except for one case, who had α^o-thalaessaemia $\alpha\alpha/--^{SEA}$. The number of Indian individuals analysed in this study might have not be representative of the Indian population in Malaysia, however it has been reported that the most common α-thalassaemia mutation in the Indian subcontinent was $\alpha\alpha/-\alpha^{3.7}$.[14]

We examined the haematological indices of the cases with heterozygous genotype and found that those with single gene deletion either (3.7 kb or 4.2kb), showed normal mean Hb (mean > 12 g/dl) with mildly low MCV (mean < 76 fL) and MCH (mean < 26 pg) (Table 2). Cases of heterozygous Hb CS ($\alpha\alpha/\alpha\alpha^{CS}$) showed normal mean Hb (mean > 12 g/dl) and MCV (mean >77 fL) with mildly low MCH (mean = 25.8 pg). The almost normal red cell indices in heterozygous Hb CS might have contributed to the low number of cases of Hb CS been diagnosed in our laboratory. The single case of heterozygous codon 59 mutation ($\alpha\alpha/\alpha\alpha^{Cd59}$) showed a normal Hb level with mildly low MCV and MCH. These findings suggest that the non-deletional α-gene abnormalities in our population gives rise to an α^+ thalassaemia trait. As expected, cases with heterozygous for the SEA gene deletions ($\alpha\alpha/--^{SEA}$) had lower Hb level (mean Hb < 12 g/dl), MCV (mean < 67 fL) and MCH (mean < 22 pg) as compared to cases with single gene deletion. Cases with homozygous and compound heterozygous for single gene deletions showed slightly lower Hb value (mean Hb < 12 g/dl), MCV (mean Hb < 72 fL) and MCH (mean < 24 pg) levels compared to cases with heterozygous single gene deletion. These findings are consistent with that described by Bain BJ 2006.[15] However, since the number of cases in our analysis was small, this might not represent the exact values.

Sixty nine (9.4%) of our cases showed the genotype consistent with the clinical diagnosis of thalassaemia intermedia. Out of 69 cases, 74% were of genotype $-\alpha^{3.7}/--^{SEA}$ followed by $-\alpha^{4.2}/--^{SEA}$ (7.3%), $\alpha\alpha^{CS}/--^{SEA}$ (4.3%), $\alpha\alpha^{CS}/\alpha\alpha^{Cd59}$ (4.3%), $\alpha\alpha^{CS}/\alpha\alpha^{CS}$ (4.3%), $-\alpha^{3.7}/\alpha\alpha^{Cd59}$ (2.9%), $\alpha\alpha^{Cd59}/\alpha\alpha^{IVS\,I\text{-}1}$ (1.4%) and $-\alpha^{3.7}/\alpha\alpha^{CS}$ (1.4%). The prevalence of three α-gene deletions ($-\alpha^{3.7}/--^{SEA}$ and $-\alpha^{4.2}/--^{SEA}$) were higher in Chinese compared to the Malays (table 2). All cases of thalassaemia intermedia had low Hb levels (between 7 – 10 g/dl) except for the single case of compound heterozygous for $-\alpha^{3.7}/\alpha\alpha^{CS}$ who showed a normal Hb level with moderately low MCV and MCH (table 2). The mild RBC parameter changes in this latter case could be accounted for by a concomitant presence of delta beta thalassaemia as suggested by the patient's high Hb F level.[16] We observed that cases of compound heterozygous $\alpha\alpha^{CS}/--^{SEA}$ appeared to have lower Hb level but higher MCV compared to the more common three α-gene deletions ($-\alpha^{3.7}/--^{SEA}$ & $-\alpha^{4.2}/--^{SEA}$) (table 2). Lower Hb level in Hb H disease involving non-deletion mutations is due to more ineffective erythropoiesis and erythroid apoptosis, while higher MCV actually due to overhydration of cells containing Hb CS.[17,18] In normal situation, production of α2-globin mRNA is higher compare to α1-globin mRNA.[2] Thus when both deletion and mutation occurs in α2 gene region; the effect is more deleterious.[2] These were observed in our patients who were homozygous for α^{CS} or compound heterozygous for α^{Cd59} and $-\alpha^{3.7}$ mutations even though two α1-globin genes were still intact. We also had a case of compound heterozygote for α^{Cd59} with IVS I nt 1 mutation in 2-genes ($\alpha^{IVS\,I\text{-}1}$) who had severe anemia and requiring regular blood transfusions.[6] In Hb Adana, a mutation in α2 codon 59, leads to production of a very unstable Hb variant.[2] Since both genes are unable to produce enough α2-globin mRNA, this leads to significantly low Hb level.

There were 10 cases (1.4%) of hydrops fetalis. Almost all except for one case showed four α-gene deletion genotype ($--^{SEA}/--^{SEA}$). The single case, who was a Malay baby, had compound heterozygosity for two α-gene deletions and Cd 59 mutation ($--^{SEA}/\alpha\alpha^{Cd59}$). This case behaved like four α-gene deletion even though only three α genes were involved.[5] Since Hb Adana is a very unstable Hb variant, with additional two α-gene deletions, the baby did not survive. Out of the nine cases with four α-gene deletion genotype, eight were Chinese and one was Malay. Higher frequency in Chinese due to higher frequency of $--^{SEA}$ genotype in Chinese compared to other races.

In terms of allelic frequency, we found that the $--^{SEA}$ allele showed the highest frequency compare to other alleles, which was 549 of 826 alleles (66.5%) followed by $-\alpha^{3.7}$ which was 220

alleles (26.6%). This was not an unexpected finding as α^0-thalaessaemia trait $\alpha\alpha/--^{SEA}$ had been shown to be relatively common in Chinese South mainland China and in other South-East Asian populations such as Thailand and Vietnam as well.[15] Xu XM *et al* showed that 48.5% α-thalassaemia carriers in Southern China were α^0-thalaessaemia trait $\alpha\alpha/--^{SEA}$.[19]

The most common α-gene abnormality in our series is $--^{SEA}$ followed by $-\alpha^{3.7}$ genotypes and the non-deletional α-thalassaemia constituting only 4% of all the α-thalassaemia cases diagnosed in our laboratory. Molecular analysis is required for definitive diagnosis of the α-thalassaemia cases and is an important prerequisite for genetic counselling. In years to come, various interactions of α-genotypes with a wide spectrum of phenotypes may be encountered, further challenging the diagnosis of thalassaemia.

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