Distribution of alpha thalassaemia in 16 year old Malaysian Students in Penang, Melaka and Sabah


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SUMMARY
Objectives: Alpha thalassaemia is wide spread in Malaysia and is a public health problem. This study aimed to describe the carrier frequencies of α-thalassaemia and its distribution among major ethnic groups in three states of Malaysia.

Methods: Educational forums were organised and study was explained to students from three schools. Students were invited to take part in the screening with parent consent. A total of 8420 adolescent students aged 16 years volunteered to participate in the study. Peripheral blood samples were analysed for complete blood counts, haemoglobin quantification and typing, and serum ferritin levels. Genomic DNA was used for screening alpha thalassaemia alleles by PCR based molecular methods.

Results: We identified seven α-globin gene defects in 341 (4.08%) students: amongst them α+ and α0-thalassaemias were detected in 232 (2.77%) and 107 (1.28%) students respectively. Genotype αα/αα was the most prevalent among sub-populations of Malay, indigenous communities of Sabah and Indian, while αα/αα deletion is more prevalent in Malaysian Chinese. It is estimated that 63 pregnancies annually are at risk of Hb Bart's hydrops fetalis.

Conclusions: We have demonstrated the prevalence and mutation patterns of α-thalassaemia in the 16 year olds in three states of Malaysia. High α-thalassaemia deletions amongst the study subjects place these carriers at an increased risk of conceiving fetuses with HbH disease and Hb Bart’s hydrops fetalis should they choose another heterozygous partner. It is therefore highly recommended to institute community screening programmes and provide prospective carriers with genetic counselling to help them make informed choices.

KEY WORDS:
α-Thalassaemia, Carrier screening, Polymerase chain reaction, Amplification refractory mutation system, Hb Bart's hydrops fetalis

INTRODUCTION
Thalassaemia syndromes are a diverse group of inherited disorders that can be characterized according to their deficient synthesis or absent production of one or more globin chains. They are classified into α, β, γ, δβ, δ and εγδ thalassaemias depending on the globin chain or chains affected. Thalassaemia is the commonest inherited monogenic disorder in Malaysia and the carrier rate for β-thalassaemia is estimated to be 4.5%. The carrier rate for the α-thalassaemias of the South East Asian deletional type has been determined to be around 4.5% in the Malaysian Chinese.

Normal individuals have four alpha genes (αα/αα) per genome, two identical genes (αα) on each chromosome 16p. Structurally, the more telomeric α-globin gene is designated the α2 gene (HBA2) and the more centromeric gene is designated the α1 gene (HBA1). The αα-thalassaemia, previously known as α-thalassaemia 1, is the complete loss of both functional α-globin genes in tandem on the same chromosome mainly caused by deletions (-α/αα). The molecular deletions resulting in α-thalassaemia 1 include the SEA, (-αα), the Thai (+-αα), and the Filipino (-αα) deletions and are presented with mild hypochromic microcytic anaemia.

It is recognised that α-thalassaemia determinants are the result of reduced α-globin gene expression, and can be caused by either deletional (-α) or non-deletional mechanisms (αα/αα or αα/αα) involving only one of the two α-globin genes. The 4.2kb deletion of DNA (leftward type, -αα) and the deletion of 3.7kb of DNA (rightward type, -αα) have been identified in our population. The -αα deletion is less common than the -αα deletion and is also present at a lower frequency in the Southeast Asian countries. These deletional forms of α-thalassaemia were previously known as α-thalassaemia 2.

Non-deletional α'-thalassaemias are rare, however several mutations have been found to be prevalent within specific populations. The cousin Spring has been found to be quite frequent in Southeast Asia. The codon 125 CTC→CCG mutation in the α2-globin gene results in unstable haemoglobin variant reported in the Chinese and Thailand population. The codon 59 mutation results in an unstable haemoglobin variant and has been reported in Indonesia and Malaysia (unpublished studies).

The total population of Malaysia, according to 2010 census was 28.3 million with the Malays and other Bumiputera...
groups accounting for about 67.4% of the population, the Chinese making up about 24.6%, the Indians about 7.3% and the other ethnic groups about 0.7% of the population. Annual population growth rate between the periods of 2000–2010 was 2%.

Inheritance with double-dose of α-thalassaemia alleles such as the SEA, (-α^0), the Thai (-α^m), and the Filipino (-α^o) deletions, result in total absence of the α-globin gene production giving rise to the Hb Barts hydrops fetalis where the affected fetuses are almost always succumb in utero.

Since Hb Barts hydrops fetalis is a public health problem and each α-thalassaemia allele has its own ethnic characteristic rate and distribution, this study was performed to estimate the carrier rate of the deletional and non-deletional forms of alpha thalassaemia mutations in our population. We studied students from three schools in Malaysia affected with hypochromic microcytosis with normal or low levels of HbA2 (<3.5%) and HbF (<1.5%) for the presence of α-thalassaemia alleles.

MATERIALS AND METHODS

In 2007 the national prevention and control program for thalassaemia was initiated with 4 main components comprising of (i) the provision of comprehensive treatment for transfusion-dependant thalassaemia cases, (ii) population screening and counselling, (iii) thalassaemia registry and (iv) health education and promotion.

As part of the programme initiative, 11,935 Malaysian adolescents were recruited into the population study, however only 8,420 adolescents (70.5%) of different ethnic backgrounds were enrolled. For various reasons which include had undergone previous testing, did not wish to know and others 3,515 students declined to participate in the study. The period of this study was one year and the students were from 3 districts in 3 different states of Malaysia. From West Malaysia, students in the states of Penang and Melaka participated in the study and from East Malaysia, Sabah students were enrolled into this study. After signed parent informed consent was obtained, 3mL of venous blood was collected into EDTA-anticoagulant from each student. Full blood count was determined for each blood sample on an automated haematology analyser, SYSMEX KX-21N (Sysmex Corporation, Kobe, Japan). Blood sample with a mean corpuscular haemoglobin level (MCH) of less than 27pg was regarded as abnormal. Further analyses were performed including HbA2 and HbF quantification using high performance liquid chromatography (VARIANT I; Bio-Rad Laboratories, Hercules, CA). Plasma ferritin levels were determined by using the chemiluminescence method. Subjects with normal or low levels of HbA2 (<3.5%) and HbF (<1.5%) where α-thalassaemia could not be ruled out, their DNA was analysed for α-globin gene mutations using polymerase chain reaction. Genomic DNA was extracted using ‘QIAamp® DNA Mini Kit’ from QIAGEN®. The single-tube multiplex PCR method described by Chong et al. was performed to genotype the common deletional forms of α-thalassaemia - two single gene deletions namely -α^0 and -α^m; and five double gene deletions, to wit the South East Asian --α^0α^0 deletion, the Filipino deletion --α^mα^m, the Thai deletion --α^mα^m, the Mediterranean double deletions --α^mα^m and --α^mα^m. For the detection of the α2 globin gene mutations the single tube multiplex amplification refractionary mutation system (ARMS) method as described by Eng et al. was used.

The common non-deletional mutations detected using this method were the termination codon TAA→CAA mutation or Hb Constant Spring, the codon 125 CTG→CCG mutation or Hb Quong Sze, codon 59 mutation (GGC→GAC), initiation codon mutation (ATG→A→G), codon 30 mutation (ΔGAC) and the codon 35 mutation (TCC→CCC). The multiplex amplification for the detection of the deletional forms of α-thalassaemia was carried out with 0.5–1.0 μg of DNA in a 50 μL solution containing 2.5U HotStarTaq DNA Polymerase in the supplied buffer (QIAGEN), 1.5mM MgCl2, 200μM each of dNTP, 1X Q-solution (QIAGEN), and primers as in Chong et al. protocol. The amplified polymerase chain reaction products were resolved by electrophoresis on a 1.0% agarose gel containing ethidium bromide. The DNA bands were visualised under UV light.

The amplification for the detection of non-deletional mutations of the α-thalassaemia globin gene was carried out with 0.5–1.0 μg of DNA in a 50 μL solution containing 2.5U HotStarTaq DNA Polymerase in the supplied buffer (QIAGEN), 1.5mM MgCl2, 200μM each of dNTP, 1X Q-solution (QIAGEN), primers in the various concentrations as in Eng et al. study. Reactions were carried out on a Eppendorf Mastercycler® (Eppendorf, Scientific, Germany) with an initial 15-minute activation/denaturation at 96°C followed by 30 cycles of denaturation at 98°C for 45 seconds, annealing at 62°C for 60 seconds, extension at 72°C for 135 seconds, and a final extension at 72°C for 5 minutes. Following amplification, 7μL of amplified PCR products were electrophoresed through 1.5% agarose gel containing ethidium bromide. The DNA bands were visualised under UV light transilluminator.

Ethical approval was obtained from the ethical review board of the Ministry of Health to perform this study.

RESULTS

A total of 8,420 16 year old students participated in the study, however only 8366 had complete test results as 54 were rejected at the genetic testing stage due to poor DNA quality post extraction. In total, 680 out of 8366 (8.1%) students were found to have MCH <27pg with iron deficiency ruled out, and had normal or low Hb F and Hb A2. Their genomic DNA was analysed for α - thalassaemia using the methods described above.

As summarised in Table I, α-thalassaemia was detected in 341 (4.08%) students of the 16 year old student population. Data revealed that 198 (2.37%) of the students were carriers of the -α^7 deletion with the -α^7/αα genotype, 88 (1.05%) were heterozygous for the SEA deletion (-α^m/αα genotype),
19 (0.23%) were carriers of Hb CS or codon 142 termination codon defect with the αCS/αα genotype, 12 (0.14%) were heterozygous for the ‒α4.2 deletion. It was observed that 15 (0.18%) students were homozygous for the ‒α3.7 deletion, ‒α3.7/‒α3.7 genotype. Interestingly, one student (0.01%) was a carrier of the codon 59 mutation αCD59/αα genotype. One student each was a compound heterozygote with ‒α3.7/‒α4.2 and ‒α3.7/αCSα, while another was homozygous for the ‒α4.2 deletion. Two (0.02%) students were heterozygous for the codon 125 mutation (αQZα/αα genotype) and 2 students were compound heterozygous for the ‒α3.7 deletion and the South East Asian deletion (‒α3.7/‒‒SEA). The Thai deletion was detected in one (0.01%) student. In 339 students no molecular defects were detected using the above test methods.

As depicted in Table II, allele frequencies for each of the α-thalassaemia in the different ethnic groups were also studied. In the Malay population 143 chromosomes (with a 95 percent confidence interval of, 123–163) had α-thalassaemia alleles. Of this, the commonest allele inherited in the Malays was the ‒α3.7 deletion allele representing a relative chromosomal frequency of 0.66. The second commonest was the ‒α4.2 deletion allele with a frequency of 0.1. The ‒α3.7 deletion allele was the next most common with a frequency of 0.07. The codon 59 mutation allele and the Thai deletion allele complimented least to the α-thalassaemia chromosome frequency, 0.007 each.

Of the 262 chromosomes studied among the Malaysian Chinese student population, 81 (95% CI, 66–96) had α-thalassaemia alleles. Commonest of them was the SEA deletion allele with a relative chromosomal frequency of 0.70, subsequent to that was the ‒α3.7 deletion allele with a gene frequency of 0.17, followed by the Constant Spring mutation allele at 0.01. The ‒α4.2 deletion allele was the next most common with a frequency of 0.04. The codon 59 mutation allele and the Thai deletion allele were detected in one (0.01%) student. In 339 students no molecular defects were detected using the above test methods.

Table I: Distribution of alpha thalassaemia determinants according to ethnicity and their population prevalences.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Ethnic groups</th>
<th>Malay, n (%)</th>
<th>Chinese, n (%)</th>
<th>Indian, n (%)</th>
<th>Sabah*, n (%)</th>
<th>Others, n (%)</th>
<th>Total with population prevalence, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‒α3.7/αα</td>
<td></td>
<td>86 (29.9)</td>
<td>13 (9.9)</td>
<td>20 (22.5)</td>
<td>75 (47.2)</td>
<td>4 (30.8)</td>
<td>198 (2.37)</td>
</tr>
<tr>
<td>‒α4.2/αα</td>
<td></td>
<td>23 (8.0)</td>
<td>57 (43.5)</td>
<td>1 (1.1)</td>
<td>4 (2.5)</td>
<td>3 (23.1)</td>
<td>88 (1.05)</td>
</tr>
<tr>
<td>αCS/αα</td>
<td></td>
<td>14 (4.9)</td>
<td>4 (3.1)</td>
<td>0 (0.0)</td>
<td>1 (0.6)</td>
<td>0 (0.0)</td>
<td>19 (0.23)</td>
</tr>
<tr>
<td>‒α3.7/αα</td>
<td></td>
<td>8 (2.8)</td>
<td>3 (2.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (7.7)</td>
<td>12 (0.14)</td>
</tr>
<tr>
<td>‒α4.2/αα</td>
<td></td>
<td>1 (0.34)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.01)</td>
</tr>
<tr>
<td>‒αCS/α4.2</td>
<td></td>
<td>4 (1.4)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>10 (6.3)</td>
<td>1 (7.7)</td>
<td>15 (0.18)</td>
</tr>
<tr>
<td>‒α4.2/α4.2</td>
<td></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.01)</td>
</tr>
<tr>
<td>‒α3.7/‒α3.7</td>
<td></td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.01)</td>
</tr>
<tr>
<td>‒α3.7/‒α4.2</td>
<td></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (1.3)</td>
<td>2 (0.02)</td>
</tr>
<tr>
<td>‒α3.7/αCD59</td>
<td></td>
<td>0 (0.0)</td>
<td>1 (0.8)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.01)</td>
</tr>
<tr>
<td>‒α3.7/αQS</td>
<td></td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.01)</td>
</tr>
<tr>
<td>αCS/α4.2</td>
<td></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (1.5)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (0.02)</td>
</tr>
<tr>
<td>Presumable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>αα/αα</td>
<td></td>
<td>150 (52.1)</td>
<td>51 (38.9)</td>
<td>67 (75.3)</td>
<td>67 (42.1)</td>
<td>4 (30.8)</td>
<td>339 (4.05)</td>
</tr>
<tr>
<td>Total with MCH&lt;27pg</td>
<td></td>
<td>288</td>
<td>131</td>
<td>89</td>
<td>159</td>
<td>13</td>
<td>680</td>
</tr>
</tbody>
</table>

* Indigenous communities of Sabah

Table II: Spectrum of alpha thalassaemic chromosomes when MCH<27pg in major ethnic groups expressed with 95% CI

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Ethnic groups</th>
<th>Malay (CI)</th>
<th>Chinese (CI)</th>
<th>Indian (CI)</th>
<th>Sabah (CI)</th>
<th>Others (CI)</th>
<th>Total tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>‒α3.7, n (CI)</td>
<td></td>
<td>94 (76.6–111.3)</td>
<td>14(6.8–21.1)</td>
<td>21 (12.6–29.4)</td>
<td>97 (89.0–113.1)</td>
<td>6 (1.8–10.2)</td>
<td>232 (205–259)</td>
</tr>
<tr>
<td>‒α4.2, n (CI)</td>
<td></td>
<td>23 (13.8–32.2)</td>
<td>57 (43.9–70.1)</td>
<td>1 (–0.9–2.9)</td>
<td>6 (1.2–10.7)</td>
<td>1 (1–6)</td>
<td>90 (72.0–107.9)</td>
</tr>
<tr>
<td>αCD59/αα</td>
<td></td>
<td>14 (6.7–21.2)</td>
<td>5 (0.7–9.3)</td>
<td>0 (–0.9–2.9)</td>
<td>1 (–0.9–2.9)</td>
<td>1 (1–6)</td>
<td>15 (7.5–22.5)</td>
</tr>
<tr>
<td>αQS/αα</td>
<td></td>
<td>1 (–0.9–2.9)</td>
<td>2 (–0.8–4.7)</td>
<td>0 (–0.9–2.9)</td>
<td>0 (–0.9–2.9)</td>
<td>1 (1–6)</td>
<td>20 (11.3–28.7)</td>
</tr>
<tr>
<td>‒α3.7/‒α3.7</td>
<td></td>
<td>1 (–0.9–2.9)</td>
<td>181</td>
<td>155</td>
<td>214</td>
<td>16</td>
<td>999</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>576</td>
<td>262</td>
<td>178</td>
<td>318</td>
<td>26</td>
<td>1360</td>
</tr>
</tbody>
</table>

The 95% confidence intervals (CI) were calculated based on the method described by Gardner et al. **

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Among the indigenous communities of Sabah, 104 chromosomes (with a 95% CI, 88–120) had α-thalassaemia alleles. The commonest α-thalassaemia inherited was the −α3.7 deletion allele with a relative chromosomal frequency of 0.93, followed by the SEA deletion at 0.06 and the Constant Spring mutation allele at 0.01.

The Malaysian Indian population contributed 23 (95% CI, 14–32) α-thalassaemia alleles for study. The commonest α-thalassaemia allele in this population was the −α3.7 deletion allele with a relative chromosomal frequency of 0.91. This was followed by the SEA deletion and −α4.2 deletion allele with a relative frequency of 0.043 each.

Relative magnitude of α0-thalassaemia and α+-thalassaemia frequencies were compared between Malay and Chinese ethnic groups using non-parametric Mann-Whitney U test. α0-Thalassaemia frequency was significantly larger in Chinese ethnic group (0.217) than its Malay counterpart (0.042) (p-value <0.0001, Mann-Whitney U test).

We have obtained data on the prevalence and mutation patterns of α-thalassaemia based on the sampling of this population. The results were stratified according to the major ethnic groups in Malaysia. The prevalence rate of α-thalassaemia in this population study was 4.08%.

A high prevalence of the −α3.7 deletion was detected in the Malaysian Malay, Malaysian Indian and the indigenous communities of Sabah population. The SEA deletion was noted to be highly prevalent in the Malaysian Chinese population, presumably because of population structure.

By ethnicity the commonest α-thalassaemia determinant detected in the Malays is the −α3.7 deletion, followed by the SEA deletion and the Constant Spring mutation. In the Chinese student population the SEA deletion was the most prevalent followed by the −α3.7 deletion and by the Constant Spring mutation. The most prevalent α-thalassaemia
The -α\textsuperscript{3.7} deletion has been identified as the most common mutation to produce a genetic disorder and is found to be prevalent in most tropical and subtropical populations studied\textsuperscript{21}. High prevalence of α-thalassaemia is noted in malaria endemic areas as the inheritance of this genetic condition has been suggested to offer a selective advantage and is believed to protect these individuals against severe anaemia\textsuperscript{22}. Severe anaemia in malaria is related to the invasion of erythrocytes by the organism causing excessive destruction of red cells. There has been some evidence indicating that the protective effect of -α/α against severe malarial-anaemia may be due to the increased erythrocyte turnover which dampens a rapid decrease in haemoglobin concentration. There is also little effect on slow decrease thus leaving time for maximum stimulation of erythropoiesis\textsuperscript{21}.

The percentage of students in this population who were heterozygous for the SEA deletion (-/αα/αα genotype) was 1.05%. Accurate detection of this deletion requires molecular techniques and is necessary as the homozygous state of α\textsuperscript{-3.7} thalassaemia results in Hb Bart’s hydrops fetalis. Prevalence of the SEA deletion is highest in the Malaysian Chinese with 43.5% positive for the deletion of the total number referred for molecular testing. In a study done in Guangdong Province in Southern China where there is a high risk for α-thalassaemia, it was noted that the SEA deletion was the most common mutation detected accounting for 48.54% of all the α-thalassaemias\textsuperscript{22}. The second most common α-thalassaemia mutation in Guangdong was the -α\textsuperscript{-2} deletion. These findings were also seen in this study.

From the proportion of -/αα/αα heterozygous genotype (88/8366) calculated and the Malaysian population growth rate of 2% per annum with 28.3 million as of 2010 census size, it could be estimated that nearly 63 pregnancies each year are at risk of Hb Bart’s hydrops fetalis syndrome (--/αα/αα). This incidence is expected to be relatively much higher in Malaysian Chinese than the -α\textsuperscript{-} recessive allele frequency is comparatively higher than other ethnic groups.

HbH disease results from the interaction of α-thal 1 (α\textsuperscript{-3.7} thalassaemia) with α\textsuperscript{-}-thalassaemia, deletional type (--/αα) or non-deletional type (--/αα/αα or --/αα). There is a severe imbalance in the globin chain synthesis resulting in the precipitation of the excess β globin chains. This results in the formation of a characteristic abnormal haemoglobin known as haemoglobin H (Hb H) or β globin tetramer or β4. Affected individuals with the deletional type of Hb H disease may have mild to moderate chronic haemolytic anaemia with the presence of detectable Hb H inclusion bodies in the peripheral red blood cells\textsuperscript{23}.

The deficit in α-globin expression in non-deletional type Hb H patients (--/αα) appears to be greater than in the deletional forms of Hb H disease (--/αα) therefore clinical phenotypes of Hb H disease found in non-deletional α-thalassaemia (--/αα) are often more severe than those caused by Hb H disease resulting from simple deletions (--/αα). In Thailand the number of symptomatic patients with Hb H disease due to non-deletional mutations appeared to be higher than those with deletional Hb H suggesting that non-deletional Hb H patients have more significant clinical symptoms and require more medical attention\textsuperscript{24}. Three non-deletional Hb H disease have been associated with the rare instances of Hb H disease hydrops fetalis syndrome. Affected fetuses have profound anaemia and findings consistent with chronic hypoxia in utero. The mutations include the codon 30 mutation (AGC), the codon 59 mutation (GGC→GAC), and the codon 35 mutation (TCC→CCC)\textsuperscript{4, 14, 15}.

Molecular analysis showed 0.26% of this student population were carriers of the non-deletional forms of α-thalassaemia. Non-deletional carriers may affect the fundamental processes of globin gene expression from mRNA transcription, splicing and protein translation with the creation of novel truncated or elongated globin peptides\textsuperscript{25}. The non-deletional mutations identified in this student population were the termination codon mutation resulting in the production of Hb Constant Spring (Hb CS), the codon 59 mutation and the codon 125 mutation resulting in the production of Hb Quong Sze (Hb QS).

The TAA→CAA mutation in the termination codon 142 of the α2 globin results in an elongated polypeptide, Hb Constant Spring. Hb CS is common in South-East Asia.\textsuperscript{6, 7, 8, 9} Another variant, Hb Pakse resulting from a TAA→TAT mutation in the termination codon of the α2 globin, also found in SEA has often been incorrectly diagnosed as Hb CS. The α\textsuperscript{α} and α\textsuperscript{α}TAT chains differ by only a single amino acid (142 glutamine in the alpha CS chain and 142 lysine in the alpha Pakse chain) and these result in abnormal slow moving haemoglobins which migrate to the same band position during Hb electrophoresis. Hence Hb Pakse has often been misidentified as Hb CS\textsuperscript{26}. A preliminary study in Thailand showed that Hb H levels were slightly higher in Hb H-Pakse disease compared with Hb H-CS disease. Since the level of Hb H in Hb H disease is thought to correlate with clinical severity therefore it is thought that Hb H-Pakse could result in a more severe disease than Hb H-CS\textsuperscript{27}. In view of these findings, confirmation of the diagnosis by molecular testing techniques should thus be carried out.

The Hb Adana [α59(E8)Gly→Asp, GGC→GAC(αα and α1)] and Hb Quong Sze [α125(H8)Leu→Pro, CTG→CCC(αα)] are examples of rare variants that lead to HbH disease or thalassaemia intermedia when associated with another α-thalassaemia defect\textsuperscript{27}. Hb H hydrops fetalis has also been diagnosed in patients with the codon 59 mutation on the α2
globin gene when associated with an α-thal 1 deletion in trans. Homozygous inheritance of the codon 59 mutation has also resulted in hydrops fetalis in 3 families in Indonesia. In an unpublished study in Malaysia, it was noted that the inheritance of the codon 142 mutation or constant spring initiation codon (ATG→A-G) in a Vietnamese girl with Hb H disease. Haemoglobin 1997, 21: 469-72.

The heterogeneity of the α-globin defects in Malaysia is possibly more varied compared with the neighbouring countries in South East Asia and this may be attributed to Malaysia being a unique blend of multi-racial communities. Interactions between the various determinants of alpha thalassaemia can produce a diverse spectrum of haematological and clinical phenotypes. In this study we have demonstrated that α-thalassaemia gene frequency in the Malaysian population as 4.08% and further described its allelic distributions in the subpopulations. This information would provide health care professionals better awareness of the possible clinical spectrum of α-thalassaemia in the population thus facilitating better management of the disease. The prevention of severe α-thalassaemia syndromes is very much dependent upon the availability of DNA testing supported by adequate genetic counselling, and targeted public awareness programmes. At risk couple counselling and molecular diagnosis of at risk populations should be made available as it is essential for the accurate diagnosis of both carrier and disease states. This will subsequently allow individuals or couples to make informed choices of their genetic risk. Thus coordinated and interactive collaboration between the relevant stakeholders is necessary to ensure the effectiveness and success of the National Prevention and Control Programme for Thalassaemia in Malaysia.

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