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 α^0–thalassaemias were detected in 232 (2.77%) and 107 (1.28%) students respectively. Genotype $-\alpha^{3.7}/\alpha\alpha$ was the most prevalent among sub-populations of Malay, indigenous communities of Sahab and Indian, while $--^{SEA}/\alpha\alpha$ 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^0–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 Barts 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 [1]. Thalassaemia is the commonest inherited monogenic disorder in Malaysia and the carrier rate for β-thalassaemia is estimated to be 4.5% [1]. 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 [1].

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 α^0-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 ($--/\alpha\alpha$) [2]. The molecular deletions resulting in α-thalassaemia 1 include the SEA, ($--^{SEA}$), the Thai ($--^{THAI}$), and the Filipino ($--^{FIL}$) 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 ($\alpha^T\alpha$ or $\alpha\alpha^T$) involving only one of the two α-globin genes. The 4.2kb deletion of DNA (leftward type, $-\alpha^{4.2}$) and the deletion of 3.7kb of DNA (rightward type, $-\alpha^{3.7}$) have been identified in our population. The $-\alpha^{4.2}$ deletion is less common than the $-\alpha^{3.7}$ deletion and is also present at a lower frequency in the Southeast Asian countries [3]. 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 [3,4,5]. Hb Constant Spring has been found to be quite frequent in Southeast Asia [6,7,8,9]. The codon 125 CTG→CCG mutation in the α2-globin gene results Haemoglobin Quong Sze or Hb QS which is a rare and highly 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

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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 [10]. Annual population growth rate between the periods of 2000-2010 was 2%.

Inheritance with double-dose of α^0-thalassaemia alleles such as the SEA, ($--^{SEA}$), the Thai ($--^{THAI}$), and the Filipino ($--^{FIL}$) 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* [11].

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 HbA_2 (<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 singed 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 HbA_2 (<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.*[12] was performed to genotype the common deletional forms of α-thalassaemia - two single gene deletions namely $-\alpha^{3.7}$and $-\alpha^{4.2}$; and five double gene deletions, to wit the South East Asian $--^{SEA}$ deletion, the Filipino deletion $--^{FIL}$, the Thai deletion $--^{THAI}$, the Mediterranean double deletions $--^{MED}$ and $-\alpha^{20.5}$. For the detection of the α2 globin gene mutations the single tube multiplex amplification refractory mutation system (ARMS) method as described by Eng *et al.* was used [13]. The common non-deletional mutations detected using this method were the termination codon TAA→CAA mutation or Hb Constant Spring [14], the codon 125 CTG→CCG mutation or Hb Quong Sze [15], codon 59 mutation (GGC→GAC) [16], initiation codon mutation (ATG→A-G) [17], codon 30 mutation (ΔGAC) [17] and the codon 35 mutation (TCC→CCC) [18].

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 $MgCl_2$, 200µM each of dNTP, 1X Q-solution (QIAGEN), and primers as in Chong *el al.* protocol[8]. The amplified polymerase chain reaction products were resolved by electrophoresis on a 1.0% agarose gel containing ethidium bromide. Electrophoresis was carried out at 80 V for 60 min. 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 $MgCl_2$, 200µM each of dNTP, 1X Q-solution (QIAGEN), primers in the various concentrations as in Eng *et al.* study [9]. Reactions were carried out on an 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 A_2. 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 $-\alpha^{3.7}$ deletion with the $-\alpha^{3.7}/\alpha\alpha$ genotype, 88 (1.05%) were heterozygous for the SEA deletion ($--^{SEA}/\alpha\alpha$ genotype),

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

Genotypes	Ethnic groups					Total with population prevalence, n (n/8366, %)
	Malay, n (%)	Chinese, n (%)	Indian, n (%)	Sabah*, n (%)	Others, n (%)	
$-\alpha^{3.7}/\alpha\alpha$	86 (29.9)	13 (9.9)	20 (22.5)	75 (47.2)	4(30.8)	198 (2.37)
$--^{SEA}/\alpha\alpha$	23 (8.0)	57 (43.5)	1 (1.1)	4 (2.5)	3 (23.1)	88 (1.05)
$\alpha^{CS}\alpha/\alpha\alpha$	14 (4.9)	4 (3.1)	0 (0.0)	1 (0.6)	0 (0.0)	19 (0.23)
$-\alpha^{4.2}/\alpha\alpha$	8 (2.8)	3 (2.3)	0 (0.0)	0 (0.0)	1 (7.7)	12 (0.14)
$--^{THAI}/\alpha\alpha$	1 (0.34)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.01)
$-\alpha^{3.7}/-\alpha^{3.7}$	4 (1.4)	0 (0.0)	0 (0.0)	10 (6.3)	1 (7.7)	15 (0.18)
$-\alpha^{3.7}/-\alpha^{4.2}$	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	1 (0.01)
$-\alpha^{4.2}/-\alpha^{4.2}$	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.01)
$-\alpha^{3.7}/--^{SEA}$	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)	0 (0.0)	2 (0.02)
$-\alpha^{3.7}/\alpha^{CS}\alpha$	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.01)
$\alpha^{CD59}\alpha/\alpha\alpha$	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.01)
$\alpha^{QS}\alpha/\alpha\alpha$	0 (0.0)	2 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.02)
Presumable $\alpha\alpha/\alpha\alpha$	150 (52.1)	51 (38.9)	67 (75.3)	67 (42.1)	4 (30.8)	339 (4.05)
Total with MCH<27pg	288	131	89	159	13	680

* Indigenous communities of Sabah

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

Alleles	Ethnic groups					Total tested 8366
	Malay	Chinese	Indian	Sabah	Others	
$-\alpha^{3.7}$, n (CI)	94 (76.6–111.3)	14(6.8–21.1)	21 (12.6–29.4)	97 (80.9–113.1)	6 (1.8–10.2)	232 (205–259)
$--^{SEA}$, n (CI)	23 (13.8–32.2)	57 (43.9–70.1)	1 (–0.9–2.9)	6 (1.2–10.7)	3 (<1–6)	90 (72.0–107.9)
$-\alpha^{4.2}$, n (CI)	10 (3.8–16.1)	3 (–0.4–6.3)	1 (–0.9–2.9)	–	1	15 (7.5–22.5)
$\alpha^{CS}\alpha$, n (CI)	14 (6.7–21.2)	5 (0.7–9.3)	–	1(–0.9–2.9)	–	20 (11.3–28.7)
$\alpha^{CD59}\alpha$, n (CI)	1 (–0.9–2.9)	–	–	–	–	1 (–0.9–2.9)
$\alpha^{QS}\alpha$, n (CI)	–	2 (–0.8–4.7)	–	–	–	2 (–0.7–4.7)
$--^{THAI}$, n (CI)	1 (–0.9–2.9)	–	–	–	–	1 (–0.9–2.9)
$\alpha\alpha$, n (CI)	433 (412.6–453.3)	181 (166.3–195.7)	155 (146.2–163.8)	214 (197.6–230.4)	16 (11–21)	999 (967.1–1030.9)
Total	576	262	178	318	26	1360

The 95% confidence intervals (CI) were calculated based on the method described by Gardner et al. [19]

19 (0.23%) were carriers of Hb CS or codon 142 termination codon defect with the $\alpha^{CS}\alpha/\alpha\alpha$ genotype, 12 (0.14%) were heterozygous for the $-\alpha^{4.2}$ deletion. It was observed that 15 (0.18%) students were homozygous for the $-\alpha^{3.7}$ deletion, $-\alpha^{3.7}/-\alpha^{3.7}$ genotype. Interestingly, one student (0.01%) was a carrier of the codon 59 mutation $\alpha^{CD59}/\alpha\alpha$ genotype. One student each was a compound heterozygote with $-\alpha^{3.7}/-\alpha^{4.2}$ and $-\alpha^{3.7}/\alpha^{CS}\alpha$, while another was homozygous for the $-\alpha^{4.2}$ deletion. Two (0.02%) students were heterozygous for the codon 125 mutation ($\alpha^{QZ}\alpha/\alpha\alpha$ genotype) and 2 students were compound heterozygous for the $-\alpha^{3.7}$ deletion and the South East Asian deletion ($-\alpha^{3.7}/--^{SEA}$). 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 $-\alpha^{3.7}$ deletion allele representing a relative chromosomal frequency of 0.66. The second commonest was the $--^{SEA}$ deletion representing at a gene frequency of 0.16. This was followed by the termination codon 142 mutation allele with a frequency of 0.1. The $-\alpha^{4.2}$ 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, 0.007each.

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 $-\alpha^{3.7}$ deletion allele with a gene frequency of 0.17, followed by the Constant Spring mutation allele at 0.06. The $-\alpha^{4.2}$ deletion allele was present at a frequency of 0.04 and this was followed by the Quong Sze mutation allele presenting at a frequency of 0.025.

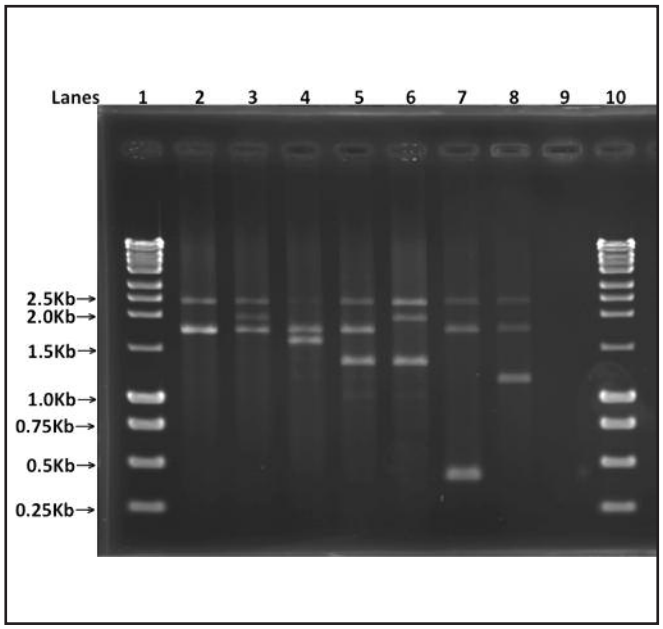

Fig. 1: Agarose gel electrophoresis of PCR products using multiplexed assays. Lanes 1 and 10 show DNA marker ladders. In lanes 2 to 8, the 2350 bp band depicts the LIS internal control band. Lane 2 shows the presence of the internal control band and the 1800 bp α2 globin gene band, lane 3 indicates presence of heterozygous -α3.7 deletion, lane 4 shows the presence of heterozygous -α4.2deletion, lane 5 denotes the presence of heterozygous --SEA deletion, lane 6 indicates the presence of the compound heterozygous state of -α3.7/--SEA, lane 7 indicates the presence of heterozygous --FIL deletion and lane 8 denotes the presence of heterozygous --THAI deletion. Lane 9 is the blank control.

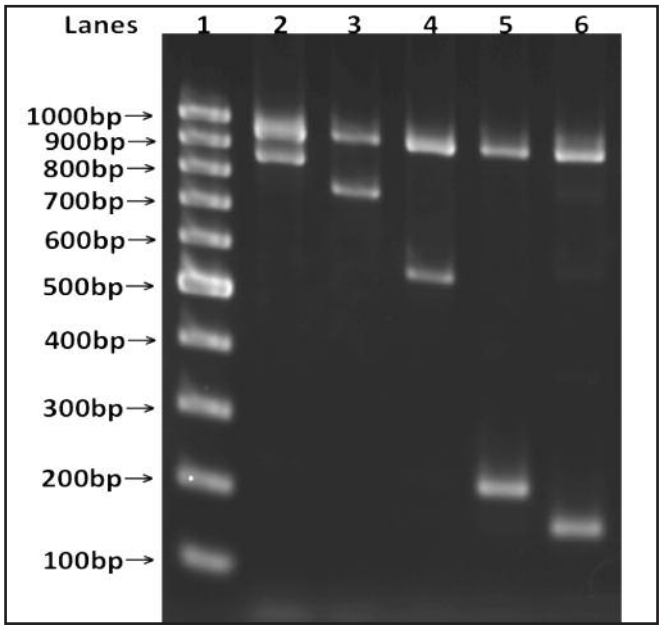

Fig. 2: Agarose gel electrophoresis of PCR products using multiplexed ARMS reaction. Lane 1 is the DNA marker ladder. In lane 2-6 the 930bp band indicates the internal control band. Lane 2 shows the presence of initiation codon mutation (ATG→A-G), lane 3 denotes presence of the codon 30 mutation (ΔGAC), lane 4 indicates the presence of codon 59 mutation (GGC→GAC), lane 5 depicts the presence of the codon 125 mutation (CTG →CCG) and lane 6 indicates the presence of the codon 142 mutation (TAA→CAA).

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 $-\alpha^{3.7}$ 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 $-\alpha^{3.7}$ deletion allele with a relative chromosomal frequency of 0.91. This was followed by the SEA deletion and $-\alpha^{4.2}$ deletion allele with a relative frequency of 0.043 each.

Relative magnitude of α^0-thalassaemia and α^+-thalassaemia frequencies were compared between Malay and Chinese ethnic groups using non-parametric Mann-Whitney U test. α^0-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).

DISCUSSION

To explore the feasibility for the implementation of a national thalassaemia screening programme, a pilot study was conducted among 16 year old Malaysian adolescents from schools in 3 districts in 3 states in Malaysia.

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 $-\alpha^{3.7}$ 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 $-\alpha^{3.7}$ 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

determinant in the Indians was the $-\alpha^{3.7}$ deletion. The population of indigenous communities of Sabah in this study group comprised of namely the Bajau, Dusun, Rungus, Murut, Sungai, Tambano, Kadazan, KadazanDusun, Suluk, Banjar, Ubian, Iranum, Jawa and Sinokadazan. Among this population, the commonest α-thalassaemia determinant detected was also the $-\alpha^{3.7}$ deletion. Although the allele frequencies of the different α-thalassaemia determinants in the different ethnic groups differed slightly from a study done previously, the spectrum of the commonest α-thalassaemia determinants in the different ethnic groups was similar to that previously described. The difference in frequencies could be attributed to the different populations studied and also due to the relatively smaller sample size in this study.

The $-\alpha^{3.7}$ 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 [19]. 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 [20]. 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 $-\alpha/\alpha\alpha$ 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 [21].

The percentage of students in this population who were heterozygous for the SEA deletion ($--^{SEA}/\alpha\alpha$ genotype) was 1.05%. Accurate detection of this deletion requires molecular techniques and is necessary as the homozygous state of α^{0}-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 [22]. The second most common α-thalassaemic mutation in Guangdong was the $-\alpha^{3.7}$ deletion. These findings were also seen in this study.

From the proportion of $--^{SEA}/\alpha\alpha$ 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 ($--^{SEA}/--^{SEA}$). This incidence is expected to be relatively much higher in Malaysian Chinese as the $--^{SEA}$ recessive allele frequency is comparatively higher than other ethnic groups.

HbH disease results from the interaction of α-thal 1 (α^{0}-thalassaemia) with α^{+}-thalassaemia, deletional type ($--/-\alpha$) or non-deletional type ($--/\alpha^{T}\alpha$ or $--/-\alpha^{T}$). 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 [23].

The deficit in α-globin expression in non-deletional type Hb H patients ($--/\alpha^{T}\alpha$) appears to be greater than in the deletional forms of Hb H disease ($--/-\alpha$) therefore clinical phenotypes of Hb H disease found in non-deletional α-thalassaemia ($--/\alpha^{T}\alpha$) are often more severe than those caused by Hb H disease resulting from simple deletions ($--/-\alpha$). 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 [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 (ΔGAC), the codon 59 mutation (GGC→GAC), and the codon 35 mutation (TCC→CCC) [16, 18, 25].

Molecular analysis showed 0.26% of this student population were carriers of the non-deletional forms of α-thalassaemia. Non-deletional mutations 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 [24]. 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 [6, 7, 8, 9]. Another variant, Hb Paksẽ 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^{CS} and α^{Pakse} 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 [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 [26]. 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(α2 and α1)] and Hb Quong Sze [α125(H8)Leu→Pro,CTG>CCG(α2)] are examples of rare variants that lead to HbH disease or thalassaemia intermedia when associated with another α-thalassaemia defect [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* [16]. Homozygous inheritance of the codon 59 mutation has also resulted in hydrops fetalis in 3 families in Indonesia [26]. In an unpublished study in Malaysia, it was noted that the inheritance of the codon 142 mutation or constant spring mutation and codon 59 mutation has resulted in infants presenting with severe anaemia as early as the first month of life.

The clinical spectrum of Hb Quong Sze in association with a double deletion SEA defect may range from mild anaemia to severe HbH disease phenotype and even to hydrops fetalis [28]. The α2 gene with the codon 125 mutation (CTG→CCG) is transcribed normally however the protein chain is rapidly degraded. The Leu→Pro mutation causes a disruption of the H helix of the alpha chain affecting the stability of the Hb tetramer [15, 29].

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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