

# Interaction of Hematological Analysis and $\alpha$ -globin Genotypes among Eligible Blood Donors

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## ABSTRACT

**Introduction:** Alpha thalassaemia is one of the haemoglobin disorders in which the carriers of alpha thalassaemia may have normal haemoglobin level and are eligible to donate blood which may bring complications. This study is to investigate the interaction of haematological parameter with  $\alpha$ -globin genotypes among eligible blood donors. **Materials & Methods:** A cohort study with 270 eligible blood donors were analysed for red cell indices. Alpha-globin ( $\alpha$ -globin) genotyping was performed for seven deletions, six point mutations and two triplications. Statistical analyses were performed to compare the  $\alpha$ -globin genotypes with haematological data. **Results:** High prevalence of  $\alpha$ -thalassaemia carriers (7.7%, 21/270) was found among blood donors. All of them did not show anaemic pictures with a normal Hb level (>12 gm/dl). Five genotypes were identified consisting of 249  $\alpha\alpha/\alpha\alpha$  (92.2%), nine  $-\alpha^{3.7}/\alpha\alpha$  (3.3%), nine  $-\alpha^{SEA}/\alpha\alpha$  (3.3%), two  $-\alpha^{4.2}/\alpha\alpha$  (0.7%) and one  $\alpha\alpha^{CS}/\alpha\alpha$  (0.4%). Different  $\alpha$ -globin genotypes showed a significant difference in RBC, MCV, MCH, MCHC, RDW, and Hct/Hb ratio ( $p < 0.05$ ) due to the different extent of  $\alpha$ -globin chain reduction. **Conclusion:** Our study concluded that by using Hb level alone is not sufficient to screen for the eligibility of blood donors. Full blood count (FBC) screening with borderline MCV and MCH levels might be able to rule out silent  $\alpha$ -thalassaemia carriers. FBC and molecular characterisation should be incorporated together to properly rule out  $\alpha$ -thalassaemia carriers.

**KEYWORDS:** red blood cell indices, alpha-thalassaemia carriers, eligible blood donor, multiplex PCR, haemoglobin

## INTRODUCTION

Alpha-thalassaemia is one of the haemoglobin disorders with a reduction or complete absence of  $\alpha$ -globin chain production. This will lead to an imbalance of the  $\alpha$ - and  $\beta$ -globin chains ratio. The excess  $\beta$ -globin chain will precipitate and cause haemolysis. Anaemic phenotype can be demonstrated in  $\alpha$ -thalassaemia individuals depending on the extent of  $\alpha$ -globin chain reduction ranging from asymptomatic, presented as “silent carrier” or “carrier” and symptomatic, presented as “intermediate” and “major”.<sup>1</sup>

Alpha-thalassaemia is inherited as an autosomal recessive pattern. When both parents are  $\alpha$ -thalassaemia carriers, there will be a 25% chance in getting an unaffected child, 50% chances in getting an asymptomatic carrier child, and 25% chances in getting an  $\alpha$ -thalassaemia major child in each pregnancy.<sup>1</sup> In Southeast Asia, thousands of infants were born with  $\alpha$ -thalassaemia intermedia (HbH) and major (Hb Barts Hydrops Fetalis) per year. The highest frequency of  $\alpha$ -thalassaemia has been seen in the Southeast Asian population, followed by Mediterranean countries, India, the Middle East, Central Asia, and Africa.<sup>1</sup> The prevalence rate of  $\alpha$ -thalassaemia in Malaysia can be up to 5%.<sup>2</sup>

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Due to the high demand for blood supply in clinical practices such as transfusions in major procedures and operations, many blood donation campaigns are organised to address the issue of inadequate blood

supply for patients. The Ministry of Health (MOH) in Malaysia emphasizes the safety of blood product transfusion.<sup>3</sup> Thus, pre-donation screening with Hb testing has been a routine practice in assuring the eligibility as blood donors other than the donors with the age of between 18 and 60 years old, bodyweight exceeding 45 kg, good physical and mental health and without any chronic medical illness or contagious diseases. However, studies have revealed that many thalassaemia traits or silent carriers are eligible blood donors due to the acceptable Hb level ranged from 12.5 to 18 g/dL and its asymptomatic features.<sup>4,5</sup> As a consequence, the thalassaemia carrier donors might be at risk of having hypoxia due to anaemia. Also, blood component especially the packed red cells of the thalassaemia carriers are not suitable for transfusion as it may cause those blood recipients who are underweight children to face deprivation of oxygen.<sup>6</sup>

Silent carriers are always being missed out from diagnosis unless molecular characterisation for  $\alpha$ -thalassaemia is conducted. The objective of this study is to elucidate the prevalence of alpha-thalassaemia among blood donors and also to investigate the interaction of red blood cell (RBC) indices with its alpha-thalassaemia genotypes at the molecular level among blood donors for proper blood donor selection.

## Methods

### *Study subjects and full blood count analysis*

Two hundred and seventy (n= 270) eligible blood donors who attended the blood donation campaign at University Tunku Abdul Rahman (UTAR), Kampar, Perak, Malaysia were recruited for this study. The study was approved by the UTAR Scientific and Ethical Review Committee (SERC) (U/SERC/97/2016). Informed consent was given before blood collection and all data were anonymized with numerical identification throughout the study. Complete blood count analysis was performed on collected peripheral blood samples using Horiba ABX Micros ES 60 (Horiba Ltd., Kyoto, Japan).

### *DNA isolation*

Three milliliters of venous blood were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers. Genomic DNA was extracted from leukocytes in

peripheral whole blood samples by using FavorPrep Blood genomic DNA extraction mini kit (Favorgen Biotech Corp, Taiwan). The quality and quantity of the extracted genomic DNA were determined using Nanophotometer UV/Vis (Implen GmbH, Munich, Germany) and genomic DNA gel electrophoresis.

### *Alpha-globin genotype analysis*

#### *Genotyping for alpha-thalassaemia triplications.*

Two alpha-thalassaemia triplications ( $\alpha\alpha\alpha^{\text{anti}3.7}$  and  $\alpha\alpha\alpha^{\text{anti}4.2}$ ) were identified using a multiplex gap polymerase chain reaction (PCR).<sup>7</sup> DNA amplification was carried out in 20  $\mu$ l reaction volume consisting of 100 ng genomic DNA, 1x PCR buffer with 2 mM  $\text{MgCl}_2$ , primers and 0.02 U of KOD *Taq* polymerase (Toyobo Co. Ltd., Osaka, Japan). The PCR cycle conditions involved initial denaturation for 15 mins at 96°C, followed by 30 cycles of 98°C denaturation for 45 secs, 64°C annealing for 90 secs, 72°C extension for 135 secs and a final extension for 5 mins at 72°C using a thermal cycler (Supercycler Trinity, Kyratex, Australia). All PCR products were electrophoresed on ethidium bromide-stained agarose gels and analyzed under bioimager (InGenius, U.K.).

#### *Genotyping of deletional alpha-thalassaemia.*

Seven most common  $\alpha$ -thalassaemia deletions ( $-\alpha^{3.7}$ ,  $-\alpha^{4.2}$ ,  $-\alpha^{20.5}$ ,  $--_{\text{SEA}}$ ,  $--_{\text{THAI}}$ ,  $--_{\text{MED}}$ ,  $--_{\text{FIL}}$ ) were identified using two modified multiplex gap PCR by amplifying across the breakpoints of each  $\alpha$ -thalassaemia deletions with the published primer sequences.<sup>8-10</sup> DNA amplification was carried out in 20  $\mu$ l reaction volume consisting of 100 ng genomic DNA, 0.5x Q-solution, primers, and 1x Qiagen multiplex PCR-master solution with 3 mM  $\text{MgCl}_2$  HotStar *Taq* DNA polymerase and dNTP mix. PCR cycle conditions involved initial denaturation for 15 min at 96°C, followed by 30 cycles of 98°C denaturation for 45 secs, 68°C annealing for 90 secs, 72°C extension for 135 secs and a final extension for 5 mins at 72°C using a thermal cycler (Supercycler Trinity, Kyratex, Australia). All PCR products were electrophoresed on ethidium bromide-stained agarose gels.

#### *Genotyping of non-deletional alpha-thalassaemia.*

Six  $\alpha$ -thalassaemia non-deletional mutations [initiation codon (ATG>AGG), codon 30 ( $\Delta$ GAG), codon 35 (TCC>CCC), codon 59 (GGC>GAC; Hb Adana), codon 125 (CTG>CCG; Hb Quong Sze) and a

termination codon (TAA>CAA; Hb Constant Spring)] were identified using a modified multiplex-PCR assay with the published primer sequences.<sup>11</sup> DNA amplification was carried out in 20 µl reaction volume consisting of 100 ng genomic DNA, 0.5x Q-solution, primers, and 1x Qiagen multiplex PCR-master solution with 3 mM MgCl<sub>2</sub> HotStar Taq DNA polymerase and dNTP mix. Cycling conditions included an initial denaturation for 12 mins at 94°C, followed by 32 cycles of 94°C denaturation for 40 secs, 64°C annealing for 20 secs, 72°C extension for 3 min and a final extension for 5 mins at 72°C using a thermal cycler (Eppendorf Mastercycler, Hamburg, Germany). All PCR products were electrophoresed on ethidium bromide-stained agarose gels.

### Statistical analysis

Kolmogorov-Smirnov (K-S), analysis of variants (ANOVA) and post hoc analysis of Fisher's Least significant difference (LSD) were performed using SPSS version 22 for normality analysis and comparison between the α-globin genotypes and red cell indices including red blood cell count (RBC), haemoglobin level (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and Hct/Hb ratio.

## RESULTS

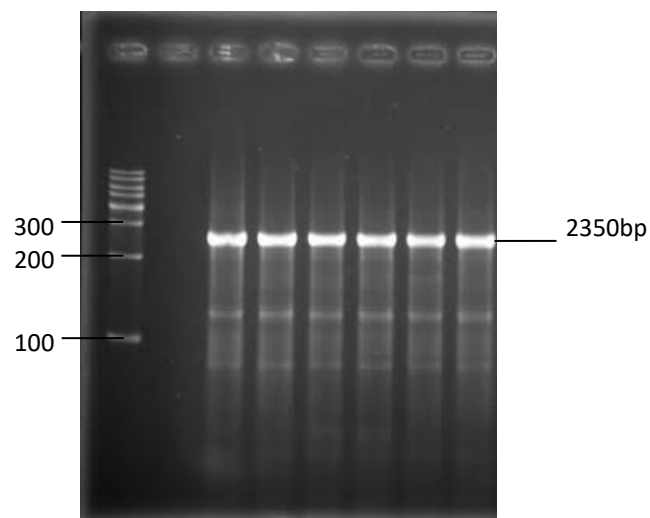
### Demographic Data

The recruited 270 blood donors were comprised of 156 males (57.78%) and 114 females (42.22%) of which 250 were Malaysian-Chinese (95.59%), followed by 3 Malays (1.11%), 15 Indians (5.56%), 1 with other

ethnicities (0.37%) and 1 with unknown ethnicity (0.37%). All the study subjects were from Malaysia either East or West Malaysia throughout the country.

### Alpha-globin genotype

Figure 1 represents the genotyping for two alpha-thalassaemia triplications (αα<sup>anti3.7</sup> and αα<sup>anti4.2</sup>). Figure 2 presents the genotyping for the detection of deletional alpha-thalassaemia and Figure 3 demonstrates the genotyping for the detection of α-thalassaemia non-deletional mutations.



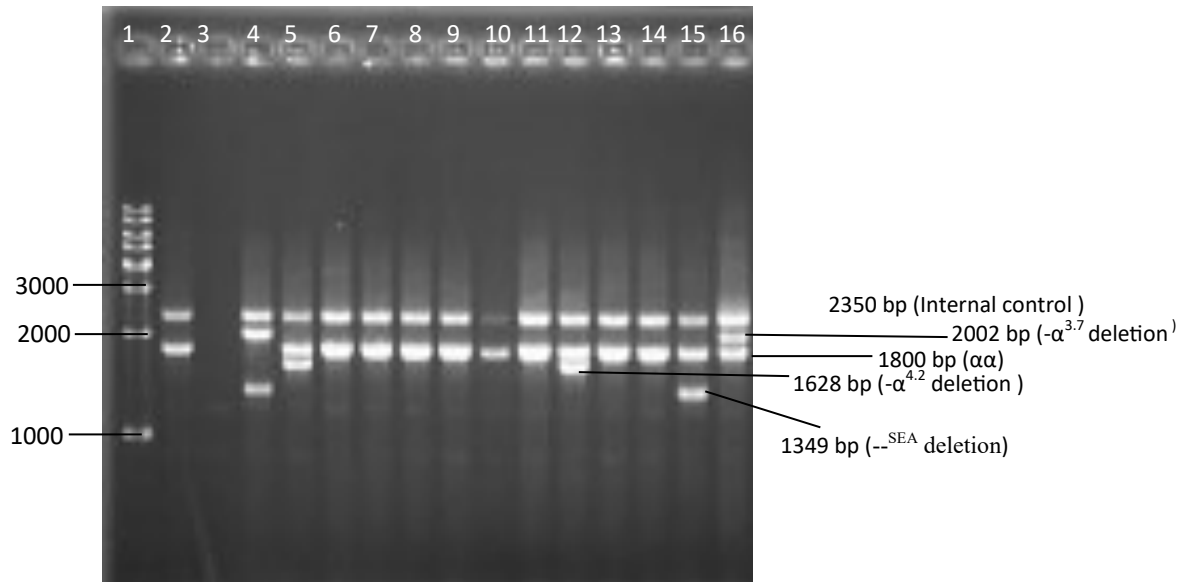
**Figure 1:** Representative gel image for the detection of two alpha triplications.

(Lane 1: 1 kb DNA ladder; Lane 2: negative control; Lane 3: normal control; Lane 4-8: samples with normal for alpha triplications)

Through molecular characterization, five genotypes were identified with 249 samples of normal α-globin genotypes (genotype 1, αα/αα), 9 samples with heterozygous -α<sup>3.7</sup> (genotype 2, αα/-α<sup>3.7</sup>) and -<sup>SEA</sup> deletion (genotype 4, αα/-<sup>SEA</sup>) respectively. The remaining were 2 samples with heterozygous -α<sup>4.2</sup> (genotype 3, αα/-α<sup>4.2</sup>) and one with heterozygous Hb constant spring (genotype 5, αα/α<sup>CS</sup>) (Table II).

**Table I:** Demographic data distribution with it α-globin genotypes

RBC indices mean ± SD (range)	Genotype 1 (αα/αα) (n=249)	Genotype 2 (αα/-α <sup>3.7</sup> ) (n=9)	Genotype 3 (αα/-α <sup>4.2</sup> ) (n=2)	Genotype 4 (αα/- <sup>SEA</sup> ) (n=9)	Genotype 5 (αα/α <sup>CS</sup> ) (n=1)	Total (n=270)
<b>Age</b>	21.7 ± 2.97 (18-43)	21.89 ± 2.15 (19-26)	22 ± 4.24 (19-25)	20.67 ± 1.50 (18-22)	22 (22)	
<b>Gender</b>						
Female	105 (38.89%)	4 (1.48%)	1 (0.37%)	3 (1.11%)	1 (0.37%)	114 (42.22%)
Male	144 (53.33%)	5 (1.85%)	1 (0.37%)	6 (2.22%)	-	156 (57.78%)
<b>Ethnicity</b>						
Chinese	231 (85.56%)	7 (2.59%)	2 (0.74%)	9 (3.33%)	1 (0.37%)	250 (92.59%)
Malays	2 (0.74%)	1(0.37%)	0	0	0	3 (1.11%)
India	14 (5.19%)	1(0.37%)	0	0	0	15 (5.56%)
Others	1 (0.37%)	0	0	0	0	1 (0.37%)
unknown	1 (0.37%)	0	0	0	0	1 (0.37%)



**Figure 2:** Representative gel image for detection of alpha single gene deletional mutation. (Lane 1:1 kb DNA ladder; Lane 2:normal control ( $\alpha\alpha/\alpha\alpha$ ); Lane 3: negative control; Lane 4: positive control ( $--^{SEA}/\alpha^{3.7}$ ); Lane 5: positive control ( $\alpha\alpha/\alpha^{4.2}$ ); Lane 6-11, 13 & 14: samples with  $\alpha\alpha/\alpha\alpha$ ; Lane 15: samples with  $\alpha\alpha/--^{SEA}$ ); Lane 12: sample with  $\alpha\alpha/\alpha^4$ ; Lane 16: sample with  $\alpha\alpha/\alpha^{3.7}$ )

### Analysis of red cell indices with alpha-globin genotypes

Kolmogorov Smirnov analysis (K-S analysis) demonstrated red cell indices and genotypes were normally distributed and preceded for parametric analysis. One-way ANOVA was conducted to compare the red cell indices on five alpha-globin genotypes as demonstrated in Table II. All the study subjects showed normal Hb level with the level >12 g/dl and

no significant difference was presented on Hb level and haematocrit (Hct) for the five alpha-globin genotypes at the  $p > .05$  level. There was a significant difference in red cell counts (RBC) on five alpha-globin genotypes at the  $p < .05$  level. Post hoc comparisons using Fisher's LSD test to compare between genotypes. RBC count for genotype 4 ( $\alpha\alpha/--^{SEA}$ ) was significantly higher than the genotype 1 ( $\alpha\alpha/\alpha\alpha$ ) ( $p=0.000$ ), genotype 2 ( $\alpha\alpha/\alpha^{3.7}$ ) ( $p=0.001$ ) and genotype 3 ( $\alpha\alpha/\alpha^{4.2}$ ) ( $p=0.015$ ).

**Table II:** Interaction of red blood cells indices with  $\alpha$ -globin genotypes

RBC indices mean $\pm$ SD (range)	Genotype 1 ( $\alpha\alpha/\alpha\alpha$ ) (n=249)	Genotype 2 ( $\alpha\alpha/\alpha^{3.7}$ ) (n=9)	Genotype 3 ( $\alpha\alpha/\alpha^{4.2}$ ) (n=2)	Genotype 4 ( $\alpha\alpha/--^{SEA}$ ) (n=9)	Genotype 5 ( $\alpha\alpha/\alpha^{CS}$ ) (n=1)	F	p-value (p < 0.05)
<b>RBC</b> ( $10^6/\text{mm}^3$ )	5.16 $\pm$ 0.68 (2.86-8.29)	5.42 $\pm$ 0.51 (4.55-6.12)	5.17 $\pm$ 0.29 (4.97-5.38)	6.46 $\pm$ 0.44 (5.74-7.00)	6.17 (6.17)	8.795	0.000**
<b>Hb</b> (g/dL)	14.71 $\pm$ 1.69 (8.6-24.8)	14.52 $\pm$ 1.33 (12.6-16.8)	13.40 $\pm$ 0.28 (13.2-13.6)	13.33 $\pm$ 0.89 (12.0-14.9)	16.30 (16.3)	2.057	0.087
<b>HCT</b> (%)	43.65 $\pm$ 5.31 (24.3-76.5)	43.71 $\pm$ 4.28 (37.0-50.8)	40.65 $\pm$ 1.63 (39.5-41.8)	42.39 $\pm$ 2.98 (37.7-47.2)	49.8 (49.8)	0.640	0.634
<b>MCV</b> (fL)	84.81 $\pm$ 5.52 (56-94)	80.56 $\pm$ 1.74 (77-83)	78.50 $\pm$ 0.71 (78-79)	65.89 $\pm$ 1.05 (64-67)	81.0 (81)	28.689	0.000**
<b>MCH</b> (pg)	28.64 $\pm$ 2.26 (17.0-33.1)	26.82 $\pm$ 0.66 (25.8-27.7)	25.90 $\pm$ 0.85 (25.3-26.5)	20.69 $\pm$ 0.65 (19.7-21.7)	26.4 (26.4)	30.508	0.000**
<b>MCHC</b> (g/dL)	33.74 $\pm$ 0.74 (30.5-35.5)	33.23 $\pm$ 0.55 (32.3-34.0)	32.95 $\pm$ 0.64 (32.5-33.4)	31.49 $\pm$ 0.50 (30.8-32.4)	32.80 (32.8)	19.739	0.000**
<b>RDW</b> (%)	14.47 $\pm$ 0.71 (13.1-16.8)	14.47 $\pm$ 0.43 (13.9-15.0)	15.05 $\pm$ 1.20 (14.2-15.9)	16.17 $\pm$ 0.59 (15.2-17.1)	14.80 (14.8)	9.792	0.000**
<b>Hct/Hb ratio</b>	2.97 $\pm$ 0.73 (2.82-3.29)	3.01 $\pm$ 0.05 (2.97-3.05)	3.03 $\pm$ 0.06 (2.51-3.55)	3.18 $\pm$ 0.05 (3.14-3.22)	3.06	20.532	0.000**

\*\* Significant different at the  $p < .05$  level

There were significant differences of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and Hct/Hb ratio on five alpha globin genotypes at the  $p < .05$  level [ $p = .000$ ].

Post hoc comparisons were performed using the Fisher's LSD test indicated the MCV's mean score for genotype 4 ( $\alpha\alpha/--^{SEA}$ ) [mean= 65.89 fL, SD=1.05] was significantly lower from genotype 1 ( $\alpha\alpha/\alpha\alpha$ ) [mean=84.81 fL, SD=5.52] with  $p=0.000$ ; genotype 2 ( $\alpha\alpha/-\alpha^{3.7}$ ) with  $p=0.000$  [mean=80.56 fL, SD=1.74] and genotype 3 ( $\alpha\alpha/-\alpha^{4.2}$ ) with  $p=0.003$  [mean=78.50 fL; SD=0.71].

For MCH, post hoc comparisons using the Fisher's LSD test indicated the mean score for genotype 4 ( $\alpha\alpha/--^{SEA}$ ) [mean=20.69 pg, SD=0.65] was significantly lower than genotype 1 ( $\alpha\alpha/\alpha\alpha$ ) [mean=28.64 pg, SD=2.26,  $p=0.000$ ], genotype 2 ( $\alpha\alpha/-\alpha^{3.7}$ ) [mean=26.82 pg, SD=0.66,  $p=0.000$ ] and genotype 3 ( $\alpha\alpha/-\alpha^{4.2}$ ) [mean=25.9 pg; SD=0.85,  $p=0.003$ ]. Similar observation was seen for MCHC, the mean score for genotype 4 ( $\alpha\alpha/--^{SEA}$ ) [mean=1.49 g/dL, SD=0.50] was significantly lower from genotype 1 ( $\alpha\alpha/\alpha\alpha$ ) with  $p=0.000$  [mean=33.74 g/dL, SD=0.74], genotype 2 with  $p=0.000$  ( $\alpha\alpha/-\alpha^{3.7}$ ) [mean=33.23 g/dL, SD=0.55] and genotype 3 with  $p=0.016$  ( $\alpha\alpha/-\alpha^{4.2}$ ) [mean=32.95 g/dL, SD=0.64].

Through the post hoc comparisons test using Fisher's LSD, it indicated the RDW and Hct/Hb ratio mean score for genotype 4 ( $\alpha\alpha/--^{SEA}$ ) was significantly higher compared to genotype 1 ( $\alpha\alpha/\alpha\alpha$ ) with  $p=0.000$ , genotype 2 ( $\alpha\alpha/-\alpha^{3.7}$ ) with  $p=0.000$  and genotype 3 ( $\alpha\alpha/-\alpha^{3.7}$ ) with  $p=0.042$  for RDW and  $p=0.008$  for Hct/Hb ratio.

## DISCUSSION

Thalassaemia is a public health concern in many regions including Malaysia where 4.5% of Malaysian-Chinese are  $\alpha^0$ -thalassaemia carriers.<sup>12</sup> This study showed a high prevalence of  $\alpha$ -thalassaemia carriers among eligible blood donor (21/270; 7.77%) consisting of four  $\alpha$ -thalassaemia genotypes including nine  $\alpha\alpha/--^{SEA}$  (9/90; 10%), nine  $\alpha\alpha/-\alpha^{3.7}$  (9/90; 10%), two  $\alpha\alpha/-\alpha^{4.2}$  (2/90; 2.2%) and one  $\alpha\alpha/\alpha\alpha^{CS}$  (1/90; 1.1%). Since our cohort of subjects is mainly of the Chinese descendant, this finding is consistent with the finding by Wee et al. (2008) with  $--^{SEA}$  and  $-\alpha^{3.7}$  as the two most common  $\alpha$ -thalassaemia mutations among the Chinese population. Southeast Asian-type deletion ( $--^{SEA}$ ) is deletion near to 20 kb of DNA at the  $\alpha$ -globin gene cluster while  $-\alpha^{3.7}$  and  $-\alpha^{4.2}$  is single  $\alpha$ -globin gene deletions of rightward 3.7 kb deletion or leftward 4.2 kb deletion with only one functional  $\alpha$ -globin gene due to recombination between Z segments or X-boxes respectively ( $-\alpha^{3.7}$ ,  $-\alpha^{4.2}$ ).<sup>12,13</sup> As the study cohort is found with more heterogeneity of



**Figure 3:** Representative gel image for detection of alpha point mutation. (Lane 1:100 bp DNA ladder; Lane 2: normal control ( $\alpha\alpha$ , 930 bp); Lane 3: negative control; Lane 4:  $\alpha\alpha/\alpha\alpha^{Cd59}$ ; Lane 5-21: samples with normal alpha-globin gene; Lane 22:  $\alpha\alpha/\alpha\alpha^{CS}$ )

the  $\alpha$ -globin gene defects compared with the other South East Asia countries due to the unique blend of multi-racial communities, thus interactions between the various  $\alpha$ -thalassaemia mutations can produce a diverse spectrum of haematological and clinical phenotypes.<sup>14</sup>

All the twenty-one cases with  $\alpha$ -thalassaemia were found with Hb level higher than 12 g/dL, fulfilling the criteria as a blood donor. Thus, this further strengthened the evidence that Hb alone is unable to rule out some of the  $\alpha$ -thalassaemia carriers as they are asymptomatic. Our study showed the eleven (n=11) samples with single-gene deletion either in heterozygous  $-\alpha^{3.7}$  or  $-\alpha^{4.2}$  were found with normal red cell indices. In this group of study subjects with only single  $\alpha$ -globin gene deletion, haemoglobin production still resumes by the remaining three functional alpha-globin genes. However, study subjects with genotype 3 ( $\alpha\alpha/-\alpha^{4.2}$ ) demonstrated lower Hb, MCV, MCH, and MCHC compared to genotype 2 ( $\alpha\alpha/-\alpha^{3.7}$ ). From the previous study,  $-\alpha^{4.2}$  deletion is reported with deletion of  $\alpha_2$ -globin gene while  $-\alpha^{3.7}$  deletion with deletion of  $\alpha_1$ -globin gene in which  $\alpha_2$ -globin gene encodes  $\alpha$ -globin chains by 2-fold more than  $\alpha_1$ -globin gene.<sup>15</sup> Thus, the deletion of the  $\alpha_2$ -globin gene will cause lesser  $\alpha$ -globin chain production and lead to a reduction of Hb amount at 30 to 35% per cell.<sup>16</sup> This may cause the production of hypochromic and microcytic red cells hence produce a lower MCV, MCH, and MCHC compared to heterozygous  $-\alpha^{3.7}$  deletion.<sup>16</sup> Normal Hb level also is shown in study subjects with genotype 4 ( $\alpha\alpha/--^{SEA}$ ) but MCV and MCH were reduced significantly compared to single-gene deletion in genotype 2 and 3. This can be attributed to the deletion of two  $\alpha$ -globin genes in  $--^{SEA}$  deletion. Two  $\alpha$ -globin gene deletion will result in a much lower  $\alpha$ -globin chain production when compared to a single  $\alpha$ -globin gene deletion. Subsequently, it will lead to the production of smaller red cell size and reduction of MCV and MCH level significantly.<sup>13, 17</sup>

According to Insiripong, Supattarabol, and Jetsricuparb (2013), the Hct/Hb ratio range among subjects with  $\alpha$ -thalassaemia is 3.3 to 4.1 which is higher than normal subjects (2.9 to 3.2) due to presence of abnormal shapes and sizes in RBCs. Our study presented with an elevation of Hct/Hb ratio in genotype 4 ( $\alpha\alpha/--^{SEA}$ ) when compared to genotype 2, 3, and 5 due to the deletion of two  $\alpha$ -globin genes. However, the reported Hct/Hb ratios were within the

range of 2.9 to 3.2 as seen in normal subjects as reported by Insiripong, Supattarabol, and Jetsricuparb. Thus, Hct/Hb ratio cannot rule out those silent  $\alpha$ -thalassaemia carriers and Hct/Hb ratio is only sensitive and specific for detection of HbH, HbH-CS or Hb Bart.<sup>22</sup>

In general,  $\alpha$ -thalassaemia resulted from point mutations may give rise to more declination in  $\alpha$ -globin chain synthesis compared to deletions as point mutations have been described to affect mRNA processing, translation, and  $\alpha$ -globin stability, thus resulting in a decreased of  $\alpha$ -globin chain production.<sup>13</sup> However, our study showed a sample with  $\alpha\alpha/\alpha\alpha^{CS}$  with normal Hb, MCV, and mild reduction of MCH level. Compared to other point mutations, Hb CS is a point mutation at Cd 142 of the  $\alpha$ -globin gene which resulted in mild Hb variant with milder clinical manifestations. When Hb CS is inherited in a heterozygous state, haemoglobins are still producing at the normal level.<sup>13</sup> Thus, the study subject with heterozygous Hb CS was presented with normal red cell indices and was also eligible as a blood donor.

The RBC count increased significantly in genotype 4 ( $\alpha\alpha/--^{SEA}$ ) and 5 ( $\alpha\alpha/\alpha\alpha^{CS}$ ), demonstrating an erythrocytosis state. Heterozygous  $--^{SEA}$  deletion ( $\alpha\alpha/--^{SEA}$ ) with deletion of both  $\alpha_1$  and  $\alpha_2$  genes will have a lower extent of  $\alpha$ -globin chains. Those extra  $\beta$ -globin chains will aggregate and form inclusion bodies within the cell. It will lead to haemolysis of RBCs in the bone marrow and extramedullary sites.<sup>18,19</sup> As the compensatory mechanism by the body for the blood with reduced oxygen-carrying capacity, erythropoietin (EPO) will increase in production by the kidney to elevate erythropoiesis.<sup>19</sup> Subsequently, it will lead to erythroid hyperplasia and elevation of mature erythrocytes in the peripheral blood.<sup>20</sup> For  $\alpha\alpha/\alpha\alpha^{CS}$ , there are cellular and membrane changes in erythrocytes with Hb Constant Spring variant. Those unstable erythrocytes will be susceptible to haemolysis and the similar compensatory mechanism in  $--^{SEA}$  deletion will occur for more RBC production.<sup>21</sup>

Among all the tested cases, none were identified as other point mutations [initiation codon (ATG>AGG), codon 30 ( $\Delta$ GAG), codon 35 (TCC>CCC), codon 59 (GGC>GAC; Hb Adana), codon 125 (CTG>CCG; Hb Quong Sze)] or triplications ( $\alpha\alpha\alpha$ ). A more significant reduction of  $\alpha$ -globin chain production is shown in  $\alpha$ -

thalassaemia with point mutation. If an individual carries an additional alpha globin gene that results in triplication ( $\alpha\alpha\alpha$ ), the  $\alpha$ -globin chain will be produced at a greater level.<sup>13</sup> Thus,  $\alpha$ -thalassaemia patients with point mutation or triplications will manifest a more severe anaemic picture and more reduction in Hb level. Thus, it is likely that these blood donors will be excluded during the screening procedure due to the low Hb level.

Transfusion of blood products from the thalassaemic patient has complications to the recipients as it may cause underweight children to be deprived of oxygen if they received the packed red cells.<sup>6</sup> Besides that, thalassaemic blood donors will tend to develop anaemia after blood donation. Our findings implied the importance of screening of  $\alpha$ -thalassaemia among blood donors. The four  $\alpha$ -thalassaemia genotypes ( $\alpha\alpha/-\alpha^{3.7}$ ;  $\alpha\alpha/-\alpha^{4.2}$ ;  $\alpha\alpha/--^{SEA}$  and  $\alpha\alpha/\alpha\alpha^{CS}$ ) reported in this study were found with normal Hb level which is more than 12 g/dl. This gives a new insight by using Hb level alone in the screening of blood donors is unable to rule out  $\alpha$ -thalassaemia silent carriers due to their normal Hb level. Different  $\alpha$ -thalassaemia mutations affect the  $\alpha$ -globin chain reduction at a different level; hence, our study revealed that red cell indices particularly RBC, MCV, MCH, MCHC, and RDW level will change accordingly.<sup>16,17</sup> Study revealed the possibility in ruling out the silent carriers especially those with borderline MCV ( $\leq 81$  fL) and MCH level ( $\leq 27$  pg). The modalities of both red cell indices and molecular characterisation will be the most ideal and proper diagnosis of  $\alpha$ -thalassaemia silent carriers. However, molecular characterisation might not be feasible especially in countries with limited resources.

## CONCLUSION

In conclusion, screening of  $\alpha$ -thalassaemia is important among blood donors. However, the current Hb screening method is not sufficient in ruling out silent  $\alpha$ -thalassaemia carriers among blood donors. Our study concluded that  $\alpha$ -thalassaemia diagnosis incorporated together with red cell indices and molecular characterisation is crucial among blood donors to prevent any missed  $\alpha$ -thalassaemia cases. However,  $\alpha$ -globin genotyping could be cumbersome and time-consuming, the study suggested at least a complete blood count should be conducted among blood donors and those with borderline MCV ( $\leq 81$  fL) and MCH level ( $\leq 27$  pg)

should precede for molecular characterisation of  $\alpha$ -thalassaemia.

## REFERENCES

1. Longo DL, Piel FB, Weatherall DJ. The  $\alpha$ -thalassemias. *New England Journal of Medicine*. 2014 Nov 13;371(20):1908-16.
2. Wee YC, Tan KL, Chow TW, Yap SF, Tan JA. Heterogeneity in  $\alpha$ -thalassaemia interactions in Malays, Chinese and Indians in Malaysia. *Journal of Obstetrics and Gynaecology Research*. 2005 Dec;31(6):540-6
3. National Blood Centre, *Transfusion Practice Guidelines for Clinical and Laboratory Personnel 3rd edition* (2008). [Online]. Available at: <<http://hsajb.moh.gov.my/versibaru/uploads/bloodbank/garispenduan2.pdf>> [Accessed 1 August 2019].
4. Azma RZ, Ainoon O, Hafiza A, Azlin I. Molecular characteristic of alpha thalassaemia among patients diagnosed in UKM Medical Centre. *The Malaysian journal of pathology*. 2014 Apr 1;36(1):27-32.
5. Bryant BJ, Hopkins JA, Arceo SM, Leitman SF. Evaluation of low red blood cell mean corpuscular volume in an apheresis donor population. *Transfusion*. 2009 Sep;49(9):1971-6.
6. Rosline H, Ahmed SA, Al-Joudi FS, Rapiaah M, Naing NN, Adam NA. Thalassaemia among blood donors at the Hospital Universiti Sains Malaysia. *Southeast Asian Journal of Tropical Medicine & Public Health*. 2006 May 1;37(3):549-52.
7. Wang W, Ma ES, Chan AY, Prior J, Erber WN, Chan LC, Chui DH, Chong SS. Single-tube multiplex-PCR screen for anti-3.7 and anti-4.2  $\alpha$ -globin gene triplications. *Clinical chemistry*. 2003 Oct 1;49(10):1679-82.
8. Chong SS, Boehm CD, Cutting GR, Higgs DR. Simplified multiplex-PCR diagnosis of common Southeast Asian deletional determinants of  $\alpha$ -thalassaemia. *Clinical chemistry*. 2000 Oct 1;46(10):1692-5.
9. Chong SS, Boehm CD, Higgs DR, Cutting GR. Single-tube multiplex-PCR screen for common deletional determinants of  $\alpha$ -thalassaemia. *Blood*. 2000 Jan 1;95(1):360-2.
- 10.
11. Tan AS, Quah TC, Low PS, Chong SS. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for  $\alpha$ -thalassaemia. *Blood*. 2001

- Jul1;98(1):250-1. <https://doi.org/10.1182/blood.v98.1.250>
12. Eng B, Patterson M, Walker L, Chui DH, Waye JS. Detection of severe nondeletional  $\alpha$ -thalassemia mutations using a single-tube multiplex ARMS assay. *Genetic testing*. 2001 Dec 1;5(4):327-9. 13.
  14. Wee YC, Tan KL, Kuldip K, Tai KS, George E, Tan PC, Chia P, Subramaniam R, Yap SF, Tan JA. Alpha-thalassaemia in association with beta-thalassaemia patients in Malaysia: a study on the co-inheritance of both disorders. *Public Health Genomics*. 2008;11(3):129-34.
  15. Farashi S, Harteveld CL. Molecular basis of  $\alpha$ -thalassemia. *Blood Cells, Molecules, and Diseases*. 2018 May 1;70:43-53.
  16. Ahmad R, Saleem M, Aloysious N, Yelumalai P, Mohamed N, Hassan S. Distribution of alpha thalassaemia gene variants in diverse ethnic populations in Malaysia: data from the Institute for Medical Research. *International journal of molecular sciences*. 2013 Sep;14(9):18599-614.
  17. Waye JS, Chui DH. The alpha-globin gene cluster: genetics and disorders. *Clinical and investigative medicine*. 2001 Apr 1;24(2):103-9.
  18. Higgs DR. The Pathophysiology and Clinical Features of Thalassemia. *Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management*. 266 (2009).
  19. Galanello R, Cao A. Alpha-thalassemia. *Genetics in medicine*. 2011 Feb;13(2):83-8. 20.
  21. Ginzburg Y, Rivella S. B-thalassemia: a model for elucidating the dynamic regulation of ineffective erythropoiesis and iron metabolism. *Blood, The Journal of the American Society of Hematology*. 2011 Oct 20;118(16):4321-30. 22.
  23. Gardenghi S, Grady RW, Rivella S. Anemia, ineffective erythropoiesis, and hepcidin: interacting factors in abnormal iron metabolism leading to iron overload in B-thalassemia. *Hematology/Oncology Clinics*. 2010 Dec 1;24(6):1089-107.
  24. Rivella S. The role of ineffective erythropoiesis in non-transfusion-dependent thalassemia. *Blood reviews*. 2012 Apr 1;26:512-5.
  25. Schrier SL, Bunyaratvej A, Khuhapinant A, Fucharoen S, Aljurf M, Snyder LM, Keifer CR, Ma L, Mohandas N. The unusual pathobiology of hemoglobin constant spring red blood cells. *Blood*. 1997 Mar 1;89(5):1762-9.
  26. Insiripong S, Supattarobol T, Jetsrisuparb A. Comparison of hematocrit/hemoglobin ratios in subjects with alpha-thalassemia, with subjects having chronic kidney disease and normal subjects. *Southeast Asian Journal of Tropical Medicine and Public Health*. 2013 Jul 1;44(4):707-11.