

# Prevalence and Disease Burden of Common Alpha Thalassaemia Deletions in Malaysian Blood Donors: A multi ethnic population

Aliza MY<sup>1</sup>, Nor Asiah M<sup>2</sup>, Manaf AA<sup>3</sup>, Chin YM<sup>1</sup>, Normi M<sup>4</sup>, Zubaidah Z<sup>1</sup> and George E<sup>5</sup>

<sup>1</sup>Hematology Unit, Cancer Research Centre,

<sup>2</sup>Biomedical Museum Unit, Medical Resource Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia.

<sup>3</sup>Deputy Vice Chancellor Office (Research and Innovation), Universiti Sultan Zainal Abidin, Terengganu Darul Iman, Malaysia

<sup>4</sup>Institute for Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

<sup>5</sup>Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia

**Abstract-** Alpha thalassaemia is common in Malaysia which comprises of Malays, Chinese, Indians and other ethnics. Therefore it is important to determine the prevalence of common alpha thalassaemia deletions that are --<sup>SEA</sup>, --<sup>THAI</sup>, --<sup>FIL</sup>, - $\alpha^{3.7}$  and - $\alpha^{4.2}$ , and to estimate the number of pregnancies each year in which the fetus would be at risk and contribute for deletional Hb Barts hydrops foetalis syndrome and deletional HbH disease in Malaysia. In this study, a cross-sectional study of 94 Malays, 129 Chinese and 7 others from Health Awareness Campaign was carried out using standard haematological analysis, multiplex PCRs and statistical analysis. Red Cell Mean Corpuscular Volume (MCV) < 80 fL was identified in 38 (16.5%) with exclusion of Indians. 17 (7.4%) of blood donors showed alpha thalassaemia deletions. 3.5% were with double gene deletion and 3.9% with single gene deletions. --<sup>SEA</sup> was seen in 7(5.4%) Chinese and 1(1.1%) Malay. - $\alpha^{3.7}$  was seen in 7 (3.0%) of 4 (4.3%) Malays, 2 (1.6%) Chinese and 1 other ethnic. - $\alpha^{4.2}$  was seen in 2 Chinese (0.9%). The projected number of pregnancies at risk and contribute for deletional Hb Barts hydrops foetalis syndrome and deletional HbH disease each year in Malaysia is 30 and 120 in the Malays, 250 and 150 in the Chinese, 640 in combination and on average is 600 and 669 respectively. The current prevalence and projected number of pregnancies at risk of deletional Hb Barts hydrops foetalis syndrome and deletional HbH disease will help to achieve a better disease management in order to lessen present alpha thalassaemia burden and to prevent much higher alpha thalassaemia birth in Malaysia.

**Index Terms-** alpha thalassaemia deletions, prevalence, disease burden, blood donor, multiplex PCR.

## I. INTRODUCTION

Alpha thalassaemias are described as genetic disorders of hemoglobin synthesis characterized by a reduction in the synthesis of alpha globin chains and affecting 5% of world's population. The alpha globin cluster consists of 5'- $\zeta$ 2- $\psi$ 1- $\psi$ 2- $\psi$ 1- $\alpha$ 2- $\alpha$ 1- $\theta$ 1-3' on chromosome 16p13.3 giving ( $\alpha\alpha/\alpha\alpha$ ) in a wild type. Absence of one alpha gene ( $\alpha\alpha/\alpha-$ ) is known as a silent carrier, absence of two ( $\alpha\alpha/--$ ), ( $-\alpha/\alpha$ ) known as alpha thalassaemia trait, absence of three ( $--/\alpha$ ) known as HbH disease with inclusions in the red cells stained with Brilliant Cresyl Blue are usually observed, and complete deletion of alpha genes ( $--/--$ )

known as Hb Barts hydrops foetalis is incompatible with life predominantly seen in Southeast Asia[1-7]. The incidence in a population reflects the balance between the premature death of homozygotes and the increased fitness in heterozygotes[8]. The most common alpha thalassaemia deletions are the Southeast Asia (--<sup>SEA</sup>), Philippines (--<sup>FIL</sup>) and Thailand (--<sup>THAI</sup>) in the Southeast Asia, the 3.7 kb (- $\alpha^{3.7}$ ) and the 4.2 kb (- $\alpha^{4.2}$ ) in the world[10-12]. Over 5% of the Filipinos are carriers for --<sup>SEA</sup> or -<sup>FIL</sup> and 14% of Northern Thailand people are carriers of --<sup>SEA</sup>[12]. In Singapore, about 6.4% Chinese (3.9%  $\alpha^0$  only in the Chinese, 2.5%  $\alpha^+$ ), 4.8% Malays, and 5.2% Indians are  $\alpha$ -thalassaemia carriers[13].

Malaysia constitutes of Malay, Chinese, Indians and other ethnics[14, 15]. The Malay is the main ethnic group and originates mainly from Malay-Polynesians (Austronesia) as Indonesia and the Philippines, and the Mon-Khmer (central Asia)[16]. Hydrops foetalis due to alpha thalassaemia was first seen in 1961 of Chinese origin and has been reported in Chinese-Indonesian, Thais and Philipino[17-20]. A survey of cord blood on healthy newborns in Kuala Lumpur showed Hb Barts in both Malays (3.2%) and Chinese (5.1%)[21]. Hb Barts was more common in Chinese than Malays and was used as surrogate marker for alpha thalassaemia[18-19, 22-23]. Currently precise diagnosis of alpha thalassaemia is carried out using DNA studies[24-28]. Hb Barts hydrops foetalis occurs in Chinese-Malaysian with 0.3:1000 births[25] and 4.5% of Chinese-Malaysian are carriers of  $\alpha^0$ . HbH disease was similarly seen in the Malays and Chinese. In contrast, antenatal diagnosis for  $\alpha$  thalassaemia reported Hb Barts hydrops foetalis mostly in the Chinese and all Chinese couples were carriers of --<sup>SEA</sup>[29-30]. Thalassaemia studies among blood donors of 91.3% (73/80) Malays found out that 30% were anaemic and all had a negative H-inclusion[31]. Deletional alpha thalassaemia burden has not been reported in Malaysia. In order to know the current carrier prevalence in our multiethnic population, carrier detection was carried out by screening blood donors. This enabled estimation of disease burden, continuous monitoring of deletional alpha thalassaemia in the country and identified carriers can be informed their risk and options regarding marriage and child-bearing[32].

II. MATERIALS AND METHODS

In conjunction with blood donation by the National Blood Service Centre in September 2004, 106 males and 132 females undergraduates came to donate blood and consent was taken for thalassaemia DNA studies. Full Blood Count was determined by ABX MICROS 60-OT18 (ABX Diagnostics, France) within 6 hours of venesection. Cell morphology assessment and Hemoglobin H inclusion test were carried out according to standard methods[33, 34]. Hb A<sub>2</sub> and F were quantified by Variant™ β-thalassaemia Short Program (BIO-RAD Laboratories, USA). Hb A<sub>2</sub> > 4.0% was the cut-off value of classical β<sup>0</sup> thalassaemia trait in Malaysia[35]. Eight samples (3%) diagnosed as β-thalassaemia trait, Hb E/AE trait, Hb S trait and suspected Hb Q were excluded from this study as coinheritor of alpha and beta thalassaemia was not included in the study. A cut-off value of MCV < 80 fL was used[2, 10, 36- 37] and 16.5% (20 males and 18 females) were subjected to DNA analysis. DNA was extracted using QIAamp® DNA Blood Midi Kit (QIAGEN, Germany). Purity and concentration were estimated using Ultrospec 3000pro (Pharmacia Biotech, USA). Polymerase Chain Reaction (PCR) was carried out using 13 primers or 8 pairs to detect --<sup>SEA</sup>, --<sup>FIL</sup>, --<sup>THAI</sup>, -α<sup>3.7</sup> or -α<sup>4.2</sup>[38] and consisted of 100ng DNA, 0.2μM each primer and QIAGEN® Multiplex PCR Kit (QIAGEN, Germany). The program was 15 minutes Hot Start at 98°C, 30 cycles of 45 seconds denaturation at 98°C, 3

minutes annealing at 64°C and 150 seconds extension for 72°C followed by 15 minutes final extension at 72°C. PCR products were electrophoresed in 2% agarose gel and ethidium bromide stained. Statistical analysis was carried out using SPSS 11.5.

III. RESULTS

In the 230, 94 (41%) were Malays, 129 (56%) Chinese and 7 (3%) of other ethnics and none from Indians. These were 19-24 years old from all 14 states in peninsular and east Malaysia. MCV and MCH of Malay (40 male and 54 female) and Chinese (58 male and 71 female) were normally distributed (p>0.05) with mean 83 fL and 28 pg, and well demonstrated in MCV of Malay male and Chinese female [39-40]. A bimodal (65 and 85 fL) MCV distribution was demonstrated in Chinese males[10]. --<sup>SEA</sup> (3.5% with 95% CI, 3.4% to 3.8%) detected were with MCV ≤ 68 fL and MCH < 22 pg and -α<sup>+</sup> (3.9% with 95% CI, 3.8% to 4.2%) with MCV ≥ 73 fL and MCH > 25.2 pg (Table 1). Total alpha thalassaemia deletions present was 17 from 5 (2.2%) Malays, 11 (4.8%) Chinese and 1 (0.4%) of other ethnics giving 7.4% (95% CI, 7.1 % to 8.3%) and were equally distributed among Malay and Chinese (Yates' corrected chi-squared, P<0.05). In the Malays, the prevalence was 5.3% (95% CI, 5.2 % to 5.9%) with 4.3% -α<sup>3.7</sup> (95% CI, 4.2% to 4.7%) and 1.1% --<sup>SEA</sup> (95% CI, 1.1% to 1.2%). In the Chinese, was 8.5% (95% CI, 8.1% to 9.7%) with 5.4% --<sup>SEA</sup> (95% CI, 5.3% to 6.0%), 1.6% -α<sup>3.7</sup> (95% CI, 1.6% to 1.7%) and -α<sup>4.2</sup> each.

Table 1: Allele frequency of alpha thalassaemia deletions detected.

Allele	MCV fL	MCH pg	Malay (n = 94)	Chinese (n = 129)	Others (n=7)	Total (n=230)
(-- <sup>SEA</sup> )	60-68	19.2-21.8	1♀ (1.1%)	7♂ (5.4%)	0	8(3.5%)
(-α <sup>3.7</sup> )	73-77	25.2-26.8	3♀ (3.2%)	1♀ (0.8%)	0	7(3.0%)
	79	26.7, 26.6	1♂ (1.1%)	1♂ (0.8%)	1♂	
(-α <sup>4.2</sup> )	76	25.5	0	1♂ (0.8%)	0	2(0.9%)
	78	26.3	0	1♀ (0.8%)	0	
Total			5 (5.3%)	11 (8.5%)	1	17(7.4%)

Malaysia population is about 28 million[41] and comprises of 58% Malays, 25% Chinese, 7% Indian and 10% of other ethnics[14-15] giving 16 million Malays and 7 million Chinese. With Malaysia's crude birth rate of 17.5 per 1000 population[15], births/year is 0.49 million with 0.3 million Malays and 0.1 million Chinese. In the Malays, the prevalence for --<sup>SEA</sup> was 1/94 meanwhile the prevalence for single α gene deletion was 4/94. In the Chinese, the prevalence for --<sup>SEA</sup> was 7/129 meanwhile the prevalence for single α gene deletion was 4/129. In both, the prevalence for --<sup>SEA</sup> was 8/223 meanwhile the prevalence for single α gene deletion was 8/223. On average, the prevalence for --<sup>SEA</sup> was 8/230 meanwhile the prevalence for single α gene deletion was 9/230. Thus the projected number of pregnancies each year in Malaysia at risk of deletional HbH disease and Hb Barts hydrops foetalis syndrome is 120 (95% CI, 116 to 125) and 30 (95% CI, 29.8 to 30.4) in the Malays, 150 (95% CI, 131 to 175) and 250 (95% CI, 195 to 319) in the Chinese, increased to 640 (95% CI, 545 to 749) in both and, 669 (95% CI, 586 to 765) and 600 (95% CI, 533 to 678) on average respectively (Table 2) [9-10, 36, 42].

Table 2: Projected number of pregnancies at risk of deletional Hb H disease and Hb Barts hydrops foetalis syndrome.

Ethnic	Hb H disease	Hb Barts hydrops foetalis syndrome
Malays	120	30
Chinese	150	250
Malay and Chinese	640	640
Average	669	600

\* The projected numbers were calculated from the values in Table 1, as follows: for Malays, 300 000 births per year X (1 ÷ 94) (4 ÷ 94) for deletional HbH disease and 300 000 births per year X (1 ÷ 94)<sup>2</sup> for deletional Hb Barts hydrops foetalis syndrome; for average, 490 000 births per year X (0.035) (0.039) for deletional HbH disease and 490 000 births per year X (0.035)<sup>2</sup> for deletional Hb Barts hydrops foetalis syndrome.

#### IV. DISCUSSION

In this study the  $--^{SEA}$ ,  $-\alpha^{3.7}$  and  $-\alpha^{4.2}$  were detected with different frequencies in Malays and Chinese-Malaysian. The most common deletion in the Malays was  $-\alpha^{3.7}$  and  $--^{SEA}$  in the Chinese-Malaysian. In the Malays, higher frequency of  $-\alpha^{3.7}$  (4.3%) was observed than  $--^{SEA}$  (1.1%). This is similar to local study of 10.7% to 2.5% respectively in pregnant mothers[30]. The lower  $--^{SEA}$  (1/94) is similar to 0.6% (9/1567) of Malay blood donors[43]. Conversely in the Chinese-Malaysian, the  $--^{SEA}$  of 5.4% (7/129) is higher than  $-\alpha^{3.7}$  (1.6%). This is consistent with 4.14% (232/5605) to 3.10% (174/5605) in Southern China newborns[9], 4.5% (81/1800) to 0.3% (6/1800) in Hong Kong high school students[12] and 15% to 10% (10/100) in Chinese-Malaysian pregnant mothers[30]. Lower  $--^{SEA}$  prevalence observed in China could be due to one child per family law practiced. In local studies, the  $--^{SEA}$  of 8.5% (22/259) was observed in the Chinese-Malaysian from  $\beta$ -thalassaemia patients with Hb A2 >3%[44] and 5.1% (6/118) in classical  $\beta$ -thalassaemia carriers with Hb A2 <4%[45]. Coinheritance of beta thalassaemia was excluded in our study. Therefore the 5.4%  $--^{SEA}$  in Chinese-Malaysian obtained is consistent with studies carried out in local and in countries in the region. Lowest  $-\alpha^{4.2}$  frequency was observed. The  $-\alpha^{4.2}$  was not detected in Malays (0/94) and consistent with also not detected (0/1567) in Malay blood donors and 1% (4/402) in Malays pregnant mothers[30]. This is consistent with study in Hong Kong high school students[36] of 0.2% (3/1800) and 0.95% (53/5605) in Southern China newborns[9]. In Chinese-Malaysian, the  $-\alpha^{4.2}$  is similar to  $-\alpha^{3.7}$  of 1.6% (2/129).

Alpha thalassaemia deletions frequency was lower in the Malays (5.3%) than the Chinese-Malaysian (8.5%). Single  $\alpha$ -globin gene deletions are common and homozygous single  $\alpha$ -globin gene deletion will give similar haematologic profiles and are not at risk of conceiving hydrops foetalis babies, however, at risk of conceiving HbH disease babies[46]. In the Malays, the higher frequency of  $-\alpha^{3.7}$  is consistent with the presence of HbH disease despite the lower frequency of  $--^{SEA}$  and very rare  $-\alpha^{4.2}$ . HbH samples encountered were also with genotype ( $\alpha^{SEA}/-\alpha^{3.7}$ ). The low  $--^{SEA}$  frequency could explain the rare incidence of Hb Barts hydrops foetalis in the Malays even though similar incidence of HbH disease as in the Chinese-Malaysian was observed. In the Chinese-Malaysian, the  $--^{SEA}$  frequency was higher and consistent with Hb Barts hydrops foetalis syndrome present in the society. The 5.4% of  $--^{SEA}$  in the Chinese-Malaysian was in accordance to 4.3% in Thailand, 4.5% in Hong Kong Chinese[36], 4.14% in Guangdong Province in Southern China[9] and 6.1% in Taiwanese Chinese[36] in which all are in the thalassaemic belt.

$--^{SEA}/--^{SEA}$  fetuses were with 10-20% Hb Portland 1 ( $\zeta_2\gamma_2$ ) and the most is Hb Bart's ( $\gamma_4$ ) which is a useless haemoglobin. Much lesser of Hb Portland 2 ( $\zeta_2\beta_2$ ) was also observed and the foetus usually survive into third trimester of gestation. The foetus succumbs to hypoxia and heart failure either in utero or shortly after birth. Serious maternal complications in pregnancies are also reported and without medical care, half were estimated to die[46]. From our estimation, the number of pregnancies each year at risk of deletional Hb Barts hydrops

foetalis is 30 in the Malays, 250 in the Chinese-Malaysian, 640 of both and reduces to 600 on average. This number was not seen as cases may not get reported due to misdiagnosis as iso-immunization or heart failure or reported as foetal ascites[20, 22, 24-25, 46]. Hydrops foetalis without  $\alpha$  thalassaemia is a common non-specific finding in a wide variety of foetal and maternal disorders[4]. Compared to the Chinese-Malaysian, the number of Malays and births/year is 2.3 (16 to 7 million) and 3 times more (0.3 to 0.1 million) respectively but the  $--^{SEA}$  prevalence is 5 times lower (1.1% to 5.4%) thus justified the 8 times lesser (30 to 250) projected number of pregnancies at risk of deletional Hb Barts hydrops foetalis. The single deletion prevalence in the Malays is similar to the Chinese (4.3% to 3.2%) thus the projected number of pregnancies at risk of deletional HbH disease is similar (120 to 150) and these were consistent with present status. Mix marriages of both will increase the number of birth per year (0.4 million) and even out both prevalence (3.6%) thus the number of pregnancies at risk of deletional Hb Barts hydrops foetalis and HbH disease (640) is similar and increases. On average, the population is 28 million and the number of births/year increases to 0.49 million thus increases (669) the number of pregnancies at risk of deletional HbH disease as  $\alpha^+$  thalassaemia was detected in other ethnics but reduces (600) the number of pregnancies at risk of deletional Hb Barts hydrops foetalis as double deletion is ethnic specific.

In conclusion, several approaches are initiated to reduce new cases of pregnancies with Hb Barts hydrops foetalis syndrome and HbH disease. These included continuous screening at antenatal clinics and at risk population as in undergraduates as carried out. Any substantial increase in our population will be detected and the affected families and  $\alpha^0$  carriers known will be recorded. This enables high risk individuals in these families are informed of genetic counselling and diagnostic services to be provided when the need arises. This will further help to control and lower the present morbidity and mortality and consequently lessen health burden. Thus an economical, comprehensive and effective management of this problem in our country will be better achieved[47-48].

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#### AUTHORS

**First Author** – Aliza Mohd Yacob, MSc. (viva voce passed), BSc, Hematology Unit, Cancer Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia, [aliza@imr.gov.my](mailto:aliza@imr.gov.my).

**Second Author** – Nor Asiah Muhamad, MBBS, MPH, Biomedical Museum Unit, Medical Resource Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia, [norasiah@imr.gov.my](mailto:norasiah@imr.gov.my)

**Third Author** – Prof. Abdul Manaf Ali, PhD, Deputy Vice Chancellor Office (Research and Innovation), Universiti Sultan Zainal Abidin, Terengganu Darul Iman, [manaf@udm.edu.my](mailto:manaf@udm.edu.my).

**Fourth Author** – Chin Yuet Meng, PhD, Hematology Unit, Cancer Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia, [chinym@imr.gov.my](mailto:chinym@imr.gov.my).

**Fifth Author** – Normi Mustapha, MSc. Institute for Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia, normi\_sbb@yahoo.com.sg

**Sixth Author** – Zubaidah Zakaria, MBBS, DCP, Head of Hematology Unit, Cancer Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia, zubaidah@imr.gov.my.

**Seventh Author** – Prof. Elizabeth George, MBBS, MRCP, FRCP, Department of Pathology, Faculty of Medicine and

Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia, elizg@medic.upm.edu.my.

**Corresponding Author** – Aliza Mohd Yacob, [aliza@imr.gov.my](mailto:aliza@imr.gov.my), 6013 3322695, 603 26162713.