Detection of Non-Deletional Alpha Thalassaemia in Hospital Tengku Ampuan Rahimah, Klang, Malaysia

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Abstract

Background: To date, more than 70 variants of non-deletional mutations associated with α-thalassaemia have been recognized and recorded, showcasing the diverse genetic manifestations of the condition.

Objective: This study is to provide data on the non-deletional alpha (α) thalassaemia cases at Hospital Tengku Ampuan Rahimah (HTAR), Klang, Selangor and to compare the hematological parameters (Hb, RBC, MCH, MCV, MCHC) of non-deletional α thalassaemia with the deletional variant.

Methods: This was a cross-sectional study involving data extraction from the HTAR hematology laboratory registry on confirmed thalassaemia cases *via* DNA analysis from January 2017 to December 2019. Hematological parameters were obtained from the laboratory database.

Results: A total of 479 α thalassaemia cases were recruited, 98 (20.5%) were non-deletional type. Among these, 75 (76.5%) were heterozygous Hb CS, 17 (17.3%) heterozygous Hb Adana, 3 (3.1%) heterozygous Hb Quong Sze (QS) and 1 (1.0%) case each of compound heterozygous Hb Constant Spring (CS) and Hb Adana ($\alpha^{CS}\alpha/\alpha^{CD59}\alpha$), compound heterozygous Hb CS and -3.7kb deletion ($\alpha^{CS}\alpha$ /-α3.7) and compound heterozygous of Hb CS and SEA deletion (αCSα/--SEA). In the single and double gene mutated groups, there was no significant difference of hematological parameters between the deletional and non-deletional groups. Among the non-deletional variants, there was a significant association between Hb, MCH and MCHC parameters and the number of mutated α gene.

Conclusion: The result can update the data of non-deletional α thalassaemia.

Keywords: *Non-deletional mutation, α thalassaemia, Hb Constant Spring, Hb Adana, Hb Quong Sze.*

INTRODUCTION

α thalassaemia, an inherited autosomal recessive disorder is the most prevalent monogenic disease affecting 5% of the global population [1]. It is most common in Mediterranean countries, South-East Asia, Africa, the Middle East and in the Indian subcontinent [2].

Being situated within the 'thalassaemia belt', Malaysia experiences a substantial incidence of α-thalassaemia, presenting a significant concern for public health. Based on a study conducted in 2012, the α-thalassaemia gene frequency in the Malaysian population was 4.08% [3]. However, a recent meta-analysis in 2020 on the α-thalassaemia prevalence rate in Southeast Asia showed that the incidence of α-thalassaemia in Malaysia was 17.3% based on five previous studies done between January 2010 and October 2019 in the country [4]. This amount is indeed high and warrants more attention as the major form of this genetic disease poses critical health complications.

α thalassaemia characterized by the reduction or absence of the αglobin gene, consists of 2 forms namely deletional and non-deletional, depending on the type of mutation involved. The number of remaining functional α globin genes determines the clinical presentation in both types of α thalassaemia. Silent carriers are asymptomatic with the presence of three functional genes whereas α thalassaemia trait with two inherited functional genes may have trivial hypochromic microcytic anemia. Hemoglobin H (HbH) disease characterized by mild to moderate hemolytic anemia, occurs when there is only one functional gene which usually results from compound heterozygous states for α+ thalassaemia and α^0 thalassaemia. When all four α globin genes are absent or dysfunctional, a very severe clinical condition called hemoglobin Bart's Hydrops fetalis or α thalassaemia major occurs, where infants almost always die in utero or briefly upon birth [2, 5]. Although the majority of cases are of the deletional type, the non-deletional variant of α thalassaemia deserves to be recognized as it causes a more severe hematological phenotype [6, 7].

To date, approximately 70 different types of non-deletional mutations of α thalassaemia have been discovered with most of them being rare. However, the cases identified exhibit mutation involving the α2 gene (HBA2) rather than the α1 gene (HBA1). In normal conditions, the expression of α2 gene is three times more than that of α1 gene. This phenomenon explains the reason for a more severe clinical picture caused by the non-deletional type of mutation [8]. Furthermore, a

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non-deletional form of mutation affecting α2 gene does not upregulate the α1 gene to compensate, as how it occurs in the deletional type of α2 gene [9].

Non-deletional mutations predominantly cause the formation of altered proteins by disrupting the essential process of globin gene expression. A few instances of termination codon mutations at position 142 include Hb Constant Spring, Hb Koya Dora and Hb Paksé. On the other hand, Hb Adana, Hb Quong Sze and Hb Evora are highly unstable variants caused by mutation affecting the protein stability. These unstable proteins deposit in the red blood cells as inclusion bodies leading to damage of the red cells. This hemolysis and inefficient erythropoiesis contribute to the pathophysiology of anemia [8, 10].

A recent study showed that in central Peninsular Malaysia, the allele frequency of non-deletional α thalassaemia is 16.25% [11]. The three most common non-deletional mutations among the Malaysians identified are Hb Constant Spring, Hb Adana and Hb Quong Sze [3, 12, 13]. A case series of α thalassaemia intermedia and hydrops fetalis due to compound heterozygosity of Hb Adana with other α thalassaemia was reported in two separate Malay families where both sets of parents were asymptomatic carriers [14].

Due to the instability of the α globin chains of the mutant genes, detection of these Hb variants by routine hemoglobin analysis remains a challenge. The gold standard for the identification of these variants is through molecular analysis. However, it is not feasible in all medical facilities. In most cases, as the carriers of α thalassaemia appear asymptomatic with normal laboratory findings, they constitute a reproductive risk for Hb H disease and Hb Bart's hydrops fetalis syndrome.

With this study, the aim is to provide data on the proportion of various forms of non-deletional α thalassaemia at Hospital Tengku Ampuan Rahimah (HTAR), Klang and their phenotypic characteristics pictured by their red blood cell indices. As previous studies have revealed distinctive prominence in the allelic distribution among the different ethnic groups in Malaysia, the samples obtained from HTAR would illustrate the population better as Klang, located on the west coast of Malaysia, and is known for its multiracial background. Comparing the hematological parameters of non-deletional α thalassaemia with its counterpart, can improve the comprehension of the non-deletional α thalassaemia characteristics. This knowledge will certainly assist in improved diagnosis and effective genetic counselling in accordance with the National Thalassaemia Prevention and Control Program launched by the Ministry of Health in 2004.

MATERIALS AND METHODS

This was a cross-sectional study conducted in the Hematology Unit of the Pathology Department, HTAR, Klang, Selangor. This medical facility is a tertiary hospital that offers hemoglobin analysis as a screening tool for thalassaemia and other hemoglobinopathies, for HTAR and some designated local health clinics.

Ethical approval for this study was obtained from both the Medical Research & Ethics Committee (MREC) of the Ministry of Health [NMRR ID: NMRR-20-2357-54391

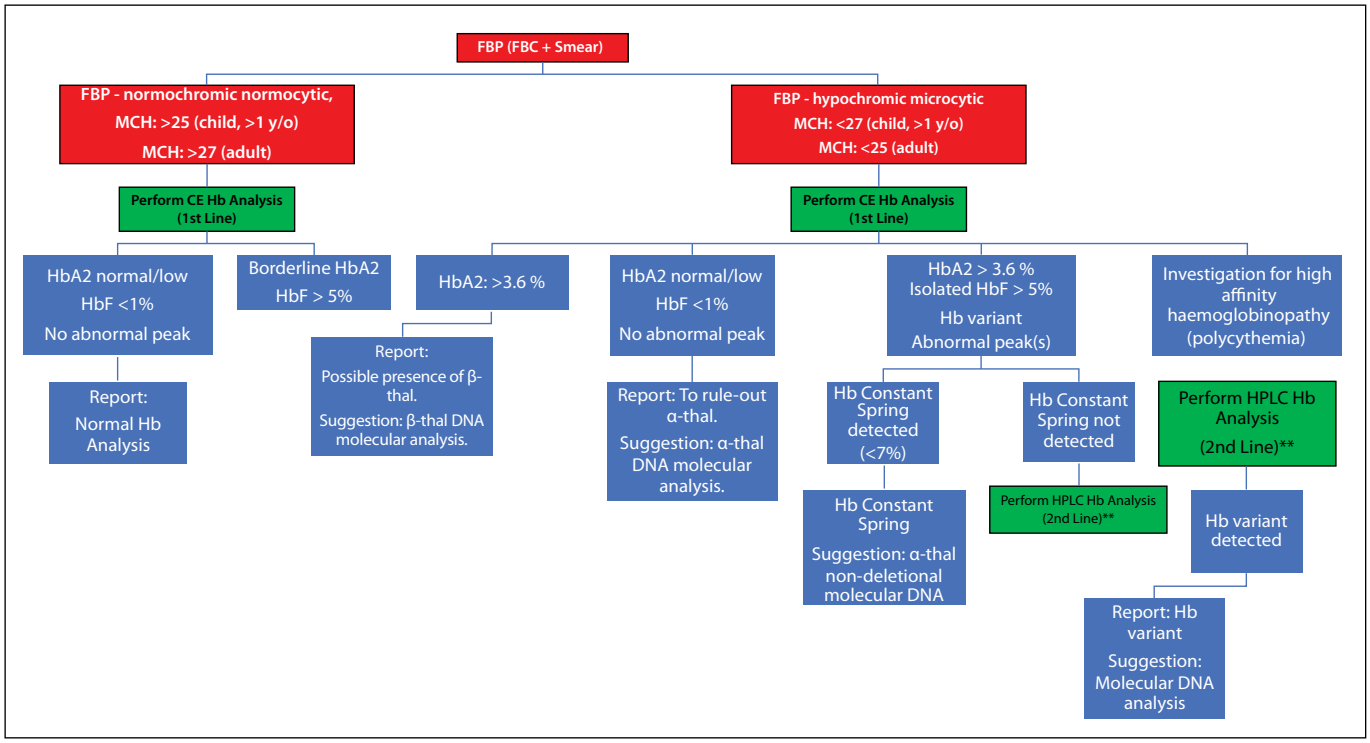

Fig. 1: Algorithm for thalassaemia screening test.

(IIR)] and the Human Research Ethics Committee USM (HREC) [JEPeM USM Code: USM/JEPeM/20120614].

Samples from HTAR itself and local health clinics with MCH of <27pg and without evidence of iron deficiency were subjected to Hb analysis in line with the National Thalassaemia Screening Programme guidelines [15]. Samples were preceded with both methods of hemoglobin analysis which were capillary electrophoresis (CE) as the first method and high-performance liquid chromatography (HPLC) as the second. Samples with abnormal hemoglobin analysis and with normal findings but in hypochromic microcytic cases were sent for molecular analysis to either Hospital Kuala Lumpur (HKL) for α globin gene analysis as shown in Fig. **(1)**.

At HKL, the molecular analysis screening panel consists of 7 deletions and 6 mutations representing common α thalassaemia determinants encountered in South-East Asia, India and the Middle East. The Multiplex Gap-Polymerase Chain Reaction (PCR) method is adopted to detect the two most common single gene deletions -α3.7 and -α4.2 and double gene deletions comprising --SEA, --FIL, --MED, --(α)20.5, --THAI. To detect non-deletional α thalassaemia mutations, multiplex amplification refractory mutation system (ARMS) PCR method is applied and those detected were initiation codon (ATG→A-G), codon 30 (GAG), codon 35 (TCC→CCC) Hb Evora, codon 59 (GGC→GAC) Hb Adana, codon 125 (CTG→CCG) Hb Quong Sze and the termination codon (TAA→CAA) Hb Constant Spring.

This study was done retrospectively involving data collection from the HTAR laboratory database of a three-year period, from 2017 to 2019. Throughout this duration, a total of 1576 samples were headed out to HKL for α globin gene analysis and out of which, 479 subjects were confirmed α thalassaemia. Purposive sampling was applied and a total of 479 subjects for a period of 3 years (2017-2019) were recruited in this study. Molecular analysis results of both deletional and non-deletional types of α thalassaemia were included in this study.

The demographic data and hematological parameters, hemoglobin (Hb), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of the subjects were retrieved from the HTAR laboratory record. The full blood count (FBC) for samples sent from local health clinics is usually done at their respective clinics and the results are not stored in the HTAR laboratory database other than two hematological parameters namely Hb and MCH, which are recorded during the reporting of hemoglobin analysis results. Subjects with incomplete data on hematological parameters were excluded from this study. Hence, a total of 324 subjects were recruited to compare hematological parameters between the deletional and the non-deletional groups of α thalassaemia.

Data were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) software, version 27. Descriptive statistics were used to represent the demographic data while median ± interquartile range (IQR) was used to represent the numerical data. Samples were divided into three different groups according to the number of affected genes: single, double and three. They were also classified into deletional and non-deletional. The statistical analysis for comparison of hematological parameters between the deletional and the non-deletional categories for the three different groups was carried out using the two-sample non-parametric Mann-Whitney U test in view of non-normal distribution. The hematological parameters were also compared among the three different groups in the non-deletional category itself using the oneway analysis of variance (ANOVA) where measurements were expressed as mean ± standard deviation (SD). For both the analysis methods, p-values less than 0.05 were considered to be statistically significant.

RESULTS

In this analysis, out of the 479 samples of α thalassaemia, 381 (79.5%) were of deletional type while the rest 20.5% represented the non-deletional mutations as shown in Table **1**. In the deletional α thalassaemia group, single gene deletions constituted more than half, comprising 213 (55.9%) samples with -3.7kb deletion (αα/-$\alpha^{3.7}$) and 17 (4.5%) samples with -4.2kb deletion (αα/-$\alpha^{4.2}$). Heterozygous for the Southeast Asian (SEA) deletion (αα/--^{SEA}) were the second most prevalent with 109 (28.6%) samples. Other types of double gene deletions observed included 16 (4.2%) samples of homozygous with -3.7kb deletion (-$\alpha^{3.7}$/-$\alpha^{3.7}$), 3 (0.8%) Filipino deletion (αα/--^{FIL}), 2 (0.5%) Thai deletion (αα/--^{THAI}) and 3 (0.8%) were compound heterozygous with -3.7kb deletion and -4.2kb deletion (-$\alpha^{3.7}$/-$\alpha^{4.2}$). Three gene deletions identified were 13 (3.4%) samples of compound heterozygous with -3.7kb deletion and SEA deletion (-$\alpha^{3.7}$/--^{SEA}), 2 (0.5%) compound heterozygous with -4.2kb deletion and SEA deletion (-$\alpha^{4.2}$/--^{SEA}) and 3 (0.8%) compound heterozygous with -3.7kb deletion and Filipino deletion (-$\alpha^{3.7}$/--^{FIL}) (**Table 1**).

In the non-deletional thalassaemia category carriers of Hb Constant Spring (α^{CS}α/αα) formed most of it with 75 (76.5%) samples. There were 17 (17.3%) samples of heterozygous Hb Adana (α^{CD59}α/αα) and 3 (3.1%) samples of heterozygous Hb Quong Sze (α^{QS}α/αα). Compound heterozygous with Hb Constant Spring and Hb Adana (α^{CS}α/α^{CD59}α) and compound heterozygous with Hb Constant Spring and -3.7kb deletion (α^{CS}α /-$\alpha^{3.7}$) showed 1 (1.0%) sample respectively. A combination of Hb Constant Spring and SEA deletion (α^{CS}α/--^{SEA}) affecting three genes was identified in 1 (1.0%) sample (**Table 1**).

From the demographic information of the non-deletional α thalassaemia (n=98), females were more apparent than

Table 1: The proportion of thalassaemia in HTAR, Klang (n = 530) from January 2017 to December 2019.

DNA Analysis result	Frequency (n)	Percentage (%)	Total n (%)
Alpha Thalassaemia			
Deletional			381 (79.5)
$-\alpha^{3.7}/\alpha\alpha$	213	55.9	
$-\alpha^{4.2}/\alpha\alpha$	17	4.5	
$\alpha\alpha/--^{SEA}$	109	28.6	
$-\alpha^{3.7}/--^{SEA}$	13	3.4	
$-\alpha^{3.7}/-\alpha^{3.7}$	16	4.2	
$\alpha\alpha/--^{THAI}$	2	0.5	
$-\alpha^{3.7}/-\alpha^{4.2}$	3	0.8	
$-\alpha^{3.7}/--^{FIL}$	3	0.8	
$\alpha\alpha/--^{FIL}$	3	0.8	
$-\alpha^{4.2}/--^{SEA}$	2	0.5	
Non-deletional			98 (20.5)
$\alpha^{CS}\alpha/\alpha\alpha$	75	76.5	
$\alpha CD59\alpha/\alpha\alpha$	17	17.3	
$\alpha^{CS}\alpha/-\alpha^{3.7}$	1	1.0	
$\alpha^{QS}\alpha/\alpha\alpha$	3	3.1	
$\alpha^{CS}\alpha/\alpha^{CD59}\alpha$	1	1.0	
$\alpha^{CS}\alpha/--^{SEA}$	1	1.0	
Total, n (%)			479 (100.0)

males with a ratio of 1.2:1. Most of the cases identified were from the age group of 13-19 years old while only one sample was from the category of 40-60 years old. The majority of the cases were Malays with 93 (94.9%) samples, followed by Chinese 4 (4.1%) and Indians 1 (1.0%)The commonest determinant inherited among the Malays was $\alpha^{CS}\alpha/\alpha\alpha$ with 72 (77.4%) cases, followed by $\alpha^{CD59}\alpha/\alpha\alpha$ with 16 (17.2%) cases, $\alpha^{QS}\alpha/\alpha\alpha$ with two (2.2%) cases and the combination alleles of $\alpha^{CS}\alpha/\alpha^{CD59}\alpha$, $\alpha^{CS}\alpha$ /$-\alpha^{3.7}$ and $\alpha^{CS}\alpha/--^{SEA}$ were at low incidence with one (1.1%) case each. Within the Chinese community, there were three (75%) cases of $\alpha^{CS}\alpha/\alpha\alpha$ and one (25%) case of $\alpha^{QS}\alpha/\alpha\alpha$ observed. Only one case of $\alpha^{CD59}\alpha/\alpha\alpha$ was identified among the Indians as shown in Table **2**.

Among the 479 samples of α thalassaemia, single gene mutation or heterozygous was identified in 220 samples. The deletional type was observed in 159 samples (72.3%) while the remaining 61 (27.7%) were of non-deletional form. The median ± interquartile range (IQR) of Hb, RBC MCV, MCH and MCHC of deletional α thalassaemia with single gene being affected were as follows: Hb 13.1 ± 1.8g/dl, RBC 5.4 ± 0.6 x 1012, MCV 75.9 ± 5.3fL, MCH 24.2 ± 1.3pg and MCHC 32 ± 1.4g/dl. Whereas, in the non-deletional counterparts, the median ± interquartile range (IQR) of Hb, RBC MCV, MCH and MCHC were Hb 13.1 ± 1.8g/dl, RBC 5.4 ± 0.6 x 1012, MCV 75.1 ± 5.9fl, MCH 24.4 ± 1.7pg and MCHC 31.9 ± 1.4g/dl. A comparison of the hematological parameters between these two groups showed no statistically significant difference (**Table 3**).

Table 3: Comparison of hematological parameters between deletional and non-deletional alpha thalassaemia with a single affected gene (n = 220).

Hematological parameters	Deletional alpha thalassaemia Median (IQR) (n = 159)	Non deletional alpha thalassaemia Median (IQR) (n = 61)	z statistic	p-value
Hb (g/dl)	13.10 (1.80)	13.10 (1.80)	-0.23	0.819
RBC (x1012/L)	5.40 (0.60)	5.40 (0.60)	-0.21	0.836
MCV (fl)	75.90 (5.30)	75.10 (5.90)	-0.30	0.764
MCH (pg)	24.20 (1.30)	24.40 (1.70)	-0.98	0.328
MCHC (g/dl)	32.00 (1.40)	31.90 (1.40)	-0.28	0.783

Note:
aMann-Whitney U test was applied.
Results are calculated by using the median ± interquartile range (IQR).
Abbreviations: Hb, hemoglobin; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration.

For the group with two gene mutations, the statistical analysis was unable to be carried out for comparison because there are only two samples of the non-deletional cases. The Hb level was lower in the non-deletional determinants (median of 11.5g/dl) compared to the deletional counterpart (median of 12.2g/dl). The RBC level was higher in the deletional cases (median of 5.9 x 1012/L) in contrast to the non-deletional variants (median of 5.4 x 1012/L). Within the deletional determinants, the median of the MCV was 65.2fl whereas the median

Table 2: Socio-demographic status among the non-deletional alpha thalassaemia (n = 98) from January 2017 to December 2019.

Socio-demographic status	Non-deletional alpha thalassaemia					
	$\alpha^{CS}\alpha/\alpha\alpha$	$\alpha^{CD59}\alpha/\alpha\alpha$	$\alpha^{CS}\alpha/-\alpha^{3.7}$	$\alpha^{QS}\alpha/\alpha\alpha$	$\alpha^{CS}\alpha/\alpha^{CD59}\alpha$	$\alpha^{CS}\alpha/--^{SEA}$
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Gender						
Male	34 (82.9)	5 (12.2)	1 (2.4)	1 (2.4)	0 (0.0)	0 (0.0)
Female	41 (71.9)	12 (21.1)	0 (0.0)	2 (3.5)	1 (1.8)	1 (1.8)
Age						
<13 years old	5 (71.4)	1 (14.3)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)
13-19 years old	60 (77.9)	13 (16.9)	1 (1.3)	3 (3.9)	0 (0.0)	0 (0.0)
20-39 years old	9 (69.2)	3 (23.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)
40-60 years old	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ethnicity						
Malay	72 (77.4)	16 (17.2)	1 (1.1)	2 (2.2)	1 (1.1)	1 (1.1)
Chinese	3 (75.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)
Indian	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)

was 72.4fl in the non-deletional variants. The MCH was 20.8pg among the deletional forms as opposed to 22.8pg in the non-deletional cases. The MCHC among the non-deletional variants was 30.2g/dl whereas it was 31.5g/dl within the deletional forms (**Table 4**).

Table 4: Comparison of hematological parameters between deletional and non-deletional alpha thalassaemia with double affected gene (n = 85).

Hematological parameters	Deletional alpha thalassaemia Median (IQR) (n = 83)	Non-deletional alpha thalassaemia Median (IQR) (n = 2)
Hb (g/dl)	12.20 (1.90)	11.45 (N/A)
RBC (x1012/L)	5.90 (0.90)	5.40 (N/A)
MCV (fl)	65.20 (5.70)	72.40 (N/A)
MCH (pg)	20.80 (1.50)	22.75 (N/A)
MCHC (g/dl)	31.50 (1.00)	30.15 (N/A)

Note:
aMann-Whitney U test was applied.
Results are calculated by using the median ± interquartile range (IQR).
Abbreviations: Hb, hemoglobin; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration.

For samples of three non-functional genes, there was a total of 19 samples, with 18 of the deletional form and only one non-deletional variant. Therefore, statistical analysis was unable to be carried out for comparison. The red cell parameters of that non-deletional sample were as follows: Hb 8.0g/dl, RBC 5.24 x 1012, MCV 46.8fL, MCH 15.3pg and MCHC 32.7g/dl. While the red cell indices of the deletional samples were in such manner: Hb 8.35 ± 0.9g/dl, RBC 5.25 ± 1.2 x 1012, MCV 55.35 ± 9.8fl, MCH 16.25 ± 1.7pg and MCHC 29.7 ± 1.5g/dl (**Table 5**).

Table 5: Comparison of hematological parameters between deletional and non-deletional alpha thalassaemia with three affected genes (n = 19).

Hematological parameters	Deletional alpha thalassaemia Median (IQR) (n = 18)	Non deletional alpha thalassaemia Median (n = 1)
Hb (g/dl)	8.35 (0.90)	8.0
RBC (x1012/L)	5.25 (1.20)	5.24
MCV (fl)	55.35 (9.80)	46.8
MCH (pg)	16.25 (1.70)	15.3
MCHC (g/dl)	29.70 (1.50)	32.7

Note:
Results are calculated by using the median ± interquartile range (IQR).
Abbreviations: Hb, hemoglobin; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration.

Among the non-deletional group itself, there was a significant association between hematological indicators of Hb, MCH, and MCHC with the number of α gene being mutated. The single gene mutation group showed the highest mean value of 13.4g/dl with a 95% confidence interval (CI) of 12.2 to 14.6g/dl, followed by a lower mean of 11.5g/dl (95% CI, 8.6 to 14.4g/dl) among the double gene mutation and the lowest mean of 8.9g/dl in the three gene mutation group. The MCH value in the three gene affected group exhibited the lowest mean value of 20.0pg compared to the double gene mutation group (mean 22.8, 95% CI, 22.7 to 22.9pg) and the single gene mutation group (mean 24.3pg, 95% CI, 22.8 to 25.8pg).

Table 6: Haematological parameters among the non-deletional alpha thalassaemia (n = 64).

Hematological parameters	Non-deletional alpha thalassaemia	Mean (SD)	Min-Max	p-value
Hb (g/dl)	Single (n = 61)	13.4 (1.2)	11.3-16.5	0.001
	Two (n = 2)	11.5 (2.9)	9.-13.5	
	Three (n = 1)	8.9 (N/A)	8.9-8.9	
RBC (x1012/L)	Single (n = 61)	5.5 (0.5)	4.6-6.5	0.138
	Two (n = 2)	5.4 (0.5)	4.1-6.7	
	Three (n = 1)	4.4 (N/A)	4.4-4.4	
MCV (fl)	Single (n = 61)	75.3 (4.0)	65.5-84.0	0.373
	Two (n = 2)	72.4 (9.5)	65.7-79.1	
	Three (n = 1)	70.8 (N/A)	70.8-70.8	
MCH (pg)	Single (n = 61)	24.3 (1.5)	20.9-28.0	0.008
	Two (n = 2)	22.8 (0.1)	22.7-22.8	
	Three (n = 1)	20.0 (N/A)	20.0-20.0	
MCHC (g/dl)	Single (n = 61)	32.1 (1.2)	29.5-35.8	0.001
	Two (n = 2)	30.2 (1.6)	29.0-31.3	
	Three (n = 1)	28.3 (N/A)	28.3-28.3	

Note:
[a]One-way ANOVA was applied.
Results are calculated by using mean ± standard deviation (SD).
Single refers to one affected gene, Two refers to two affected genes, Three refers to three affected genes.
Abbreviations: Hb, hemoglobin; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration.

For the MCHC parameter, the single gene mutation group revealed the highest mean value of 32.1g/dl (95% CI, 30.9 to 33.3g/dl), followed by a mean value of 30.2g/dl (95% CI, 28.6 to 31.8g/dl) in the double gene mutation group with the lowest mean value of 28.3g/dl in the three genes affected group. However further analysis using the post-hoc Tukey test could not be conducted for the Hb, MCH and MCHC indicators as the numbers were too small to compare. No significance was observed among the RBC and MCV parameters with the number of α gene mutation (**Table 6**).

DISCUSSION

The ultimate goal of this study was to characterize the frequency and hematological parameters of non-deletional forms of α thalassaemia cases in HTAR, Klang. Incidental findings of abnormal red cell parameters, individuals with a family history of thalassaemia, symptomatic hypochromic microcytic anemia, form 4 students as part of the National thalassaemia screening program and prenatal diagnosis for thalassaemia carrier couples are the types of cases received for thalassaemia screening after excluding cases with iron deficiency anemia.

A total of 1576 samples were outsourced to HKL for molecular analysis of α globin gene, out of which 479 samples were confirmed αthalassaemia. The formal results from HKL are usually sent electronically to the

HTAR laboratory registry, which then are printed and dispatched to the respective clinics, wards and local health clinics. In this study, 98 out of 479 (20.5%) cases showed results consistent with non-deletional α thalassaemia. This makes the ratio of non-deletional to deletional α thalassaemia at HTAR 1:3.9, consistent with the fact that the deletional form of α thalassaemia is more prevalent [16].

From this analysis of the non-deletional types of α thalassaemia, the majority of them were of single gene mutation variants, amounting to 95 (96.9%) cases. The most common type was heterozygous Hb Constant Spring (αCSα/αα) with 75 (78.9%) cases, predominantly among the Malay population with 72 (96%) cases. This finding was consistent with the previous study done by Rahimah *et al.* in 2012 and 2013 [3, 12]. Three other cases were detected in the Chinese (4%). Hb Constant Spring is caused by point mutation at the termination codon of α2 globin gene (HBA2), TAA→CAA. It results in the insertion of a glutamine molecule in the stop codon leading to the production of an unstable αglobin chain with 172 instead of 141 amino acid residues [17].

Hb Adana was the second largest type of non-deletional α thalassaemia determinant with 17 (17.3%) cases identified. This finding of Hb Adana being the second most prevalent non-deletional variant is similar to a previous study done by Rahimah *et al.* in 2012 [12]. Sixteen cases (94.1%) were seen in the Malay population while one case was among the Indians (5.9%). This highly unstable variant results from a mutation caused by Glycine→Aspartate substitution at codon 59 on the α1 or α2 globin gene. It was first described in Adana, Turkey involving the α1 globin gene in 1993. However, the cases that have been reported in Malaysia are similar to those in Indonesia which affects the α2 globin gene [18]. The prevalence rate in Indonesia is 16% while in Malaysia, the rate ranges from 1% to 21.4% [19].

Three cases (3.1%) of Hb Quong Sze (Hb QS) were identified in this study, where two cases were among the Malays and one in the Chinese. This finding of low incidence observed only in the Malay and Chinese agreed with the previous study done by Rahimah *et al.* in 2013. It was also reported in that same study that the incidence rate of Hb QS was seven times higher among the Malaysian Chinese compared to the Malays [12]. In another study conducted by Rahimah *et al.* in 2012 among 16-year-old students in Penang, Melaka and Sabah, 2 cases of Hb QS were identified in the Chinese only [3]. This less prevalent type of non-deletional mutation arises from an alteration at codon 125 on the α2 globin gene, in which amino acid proline is substituted by leucine residue. This unstable hemoglobin variant has been reported mostly in Southern China and Thailand [8] and has been observed to be one of the main alleles responsible for non-deletional Hb H (β4) disease among the Chinese population [20].

One case of compound heterozygote for non-deletional variant $\alpha^{CS}\alpha/\alpha^{CD59}\alpha$ and the other was compound heterozygous $\alpha^{CS}\alpha/-\alpha^{-3.7}$ were detected in this study. Both cases were Malays. There was only one HbH disease with double heterozygosity for α0 thalassaemia and a non-deletional mutant, $\alpha^{CS}\alpha/--^{SEA}$ seen in a Malay. Although there was only one case detected, previous studies have proven that Hb CS was the most frequent type of non-deletional mutation causing non-deletional Hb H disease [21].

In this study, the hematological parameters were compared between the deletional and the non-deletional α thalassaemia determinant by categorizing them into groups based on the number of affected genes. In the single gene-affected group, both groups showed similar Hb and RBC levels with normal hemoglobin levels (median of 13.1g/dl) and raised RBC levels (median 5.4 x 1012/L). The MCV and MCH levels were low in both groups. In the non-deletional group, the MCV was lower (median 75.1fl) compared to the deletional group (median 75.9fl), and the MCH in the deletional group was slightly lower (median 24.2pg) than the non-deletional group (median 24.4pg). They both exhibited normal MCHC levels. However, all the parameters were not significantly different between both groups. These findings suggest that non-deletional cases with one non-functional gene in our study portray the characteristics of α+ thalassaemia trait. This outcome agrees with a previous study done in 2014 by Azma *et al.* on molecular characteristics of α thalassaemia among patients diagnosed in UKM Medical Centre [22].

In the second group with two gene-affected samples, a comparison of the hematological characteristics between the deletional and non-deletional cases showed that Hb, RBC and MCHC of the non-deletional samples were lower than that of the deletional forms. Meanwhile, the MCV and MCH of the non-deletional forms were higher in comparison to their counterparts. The Hb level was lower by 0.7g/dl in the non-deletional determinants (median of 11.5g/dl) compared to the deletional variant (median of 12.2g/dl). The RBC level was higher at 0.5 x 1012/L in the deletional cases. Both the MCV and MCH were lower among the deletional cases in comparison to the non-deletional forms. Within the deletional determinants, the median of the MCV was 65.2fl and the MCH was 20.8pg whereas among the non-deletional forms, the MCV median was 72.4fl and the MCH was 22.8pg. The MCHC among the non-deletional variants was 30.2g/dl whereas it was 1.3g/dl higher within the deletional forms. All the parameters being compared in this group with 2 non-functional genes did not exhibit any significant difference between both the deletional and non-deletional determinants. Besides, since the number of cases in the non-deletional type was only two compared to 83 deletional cases, this might not represent the exact values.

In three genes affected group, there were 18 samples with the deletional type compared to only one sample of the non-deletional form. The characteristics of non-deletional Hb H disease with this genotype $\alpha^{CS}\alpha/--^{SEA}$ showed a lower Hb, RBC, MCV and MCH with higher MCHC as opposed to the deletional Hb H disease determinants. The lower Hb and RBC findings were consistent with a study conducted by Sakorn *et al.* in 2018 on hematological analysis in Thai samples with deletional and non-deletional Hb H disease. In contrast, they noted a higher MCV, MCH and a lower MCHC as opposed to our results [6]. Different study showed that the MCV and MCH among the non-deletional variants were higher compared to their deletional counterparts in Hb H disease [23]. The lower hemoglobin level in the non-deletional type of Hb H disease is attributed to the more ineffective erythropoiesis and hemolysis, whereas the higher MCV is caused by overhydration of cells with Hb CS and the presence of reticulocytes in the peripheral blood [24]. This discrepancy in this study is probably due to the very small sample size of the non-deletional Hb H and does not represent the precise value for comparison.

We also studied the characteristics of the red cell indicators among the different non-deletional α thalassaemia determinants itself, by comparing between groups of non-functional genes. The median of Hb level in the single affected gene group was 13.4g/dl, which lowered to 11.5g/dl in the double affected gene group and the least 8.9g/dl in the non-deletional Hb H disease. Likewise, the MCH also reduced as the number of affected genes increased, from 24.3pg in the single affected gene group, to 22.8pg in the double gene affected group and to 20.0pg in the three genes affected group. The MCHC indicator also showed a reduction with the increment of the affected genes. These three parameters showed significant association with the level of α gene mutation. Given the limited sample size, further analysis using the post-hoc Tukey test was not done. Although both the RBC and MCV indicators showed a reduction with the increasing number of affected genes, this analysis exhibited no significant association of these two parameters with the number of mutated genes.

This analysis faced a few significant limitations. Firstly, a limited sample size of the non-deletional α thalassaemia variant due to its scarcity caused accurate comparison with the deletional determinants to be unfeasible. Second, some of the referred samples from local health clinics had no results of the hematological indicators likely due to misplacement. Finally, patients with negative for the 13 α thalassaemia determinant screening panel during molecular analysis, were assumed to be probable αα/αα genotype with no further investigation for other possible genotypes.

CONCLUSION

In conclusion, this study's result on prevalence of the non-deletional determinants of α thalassaemia corresponds with the previous few studies done in Malaysia with Hb CS being the highest incidence followed by Hb Adana and Hb Quong Sze. There is a statistically significant association between Hb, MCH and MCHC parameters and the number of mutated α genes among the non-deletional α thalassaemia forms. However, this study did not determine any statistically significant difference between the hematological values of the non-deletional mutations as compared to the deletional mutations that had one gene defect. Meanwhile, the comparison in the two and three mutated gene groups was unlikely due to the small sample size. Hoping for more future studies to be conducted on non-deletional α thalassaemia determinants, especially those with double and three affected genes to comprehend their characteristics better and to assist in accurate diagnosis for adequate genetic counselling in the prevention of severe α thalassaemia syndromes.

ETHICAL APPROVAL

Ethical approval for this study was obtained from both the Medical Research & Ethics Committee (MREC) of the Ministry of Health [NMRR ID: NMRR-20-2357-54391 (IIR)] and the Human Research Ethics Committee USM (HREC) [JEPeM USM Code: USM/JEPeM/20120614]. All procedures performed in studies involving human participants were following the ethical standards of the institutional and/ or national research committee and the Helsinki Declaration.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

The data presented in this study are available on request from the corresponding author.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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AUTHORS' CONTRIBUTION

Conceptualization, V.K. and R.B.; writing—original draft preparation, V.K., and R.B.; writing—review and editing, S.M.Z, Z.Z. M.F.J., and R.B. All authors have read and agreed to the published version of the manuscript.

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