Original article

Molecular and haematological characterization of deletional alpha thalassemia in northeastern Malaysia

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Abstract:

Background: Alpha (α) thalassemia is an inherited condition that often cause public health problem in Malaysia. Thalassemia screening program plays extremely important roles for early detection. Therefore, it is important to accurately characterized molecular and hematologic parameters to determine the carrier genotype and prevent thalassaemia major or intermedia offspring. ***Objective:*** The objective of this study is to detect α-globin gene deletions and evaluate the haematological parameters among deletional α thalassemia patients in Hospital Universiti Sains Malaysia, Northeastern Malaysia. ***Result***: 71 (33.2%) out of of 214 samples in this study, were detected with α thalassaemia deletional type. South-East Asian (SEA) (50.7%) was most common type α deletional thalassaemia detected. There was a significant difference between the median of Hb, MCV and MCH level of patients with and without α deletion. ***Conclusion:*** This study highlighted the importance of hematological parameter as well as Hb analysis to be used as a guide before proceed with molecular method in establishing a definitive diagnosis for proper management.

Keywords: deletional alpha thalassemia;hematological parameters; screening tool; molecular

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Introduction:

In Malaysia, α thalassemia is a public health concern.[1] α-gene deletion carriers usually presented with or without anemia and mild red blood cell (RBC) microcytosis. However, it is essential to detect α -thalassemia to confirm the cause of microcytosis and to preventive unnecessary repeated test and/or lengthy iron treatment. MCH is a better characteristic compared to other red cell indices α-thalassemia diagnosis.[2] In α thalassemia, the hematological parameters showed significant correlation with the number of affected α globin genes.[3,4] The severity of microcytosis was correlated with the type of α gene deletion, eg: genotype --/αα is associated with the

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lowest MCV, followed by the -α/-α, and then the -α/αα, while the RBC counts showed vice versa.[5] However, in certain cases, the correlation between the number of gene deletion and the degree of microcytosis is not clearly seen and cannot be used as guide for proper diagnosis. Effective screening parameters with hematological parameters cut-off value to differentiate α thalassemia from other condition is important to make the preventive program a success and for correctly select the patients for molecular testing of α thalassemia to reduce the unnecessary cost of diagnostic investigations.

So far in Asia, approximately 7 types of deletions in the α -globin gene have been identified within the Southeast Asian populations which include [-α3.7,-α4.2,--SEA,--FIL,--MED,-(α)20.5, and --THAI].[6]Malaysia is a multi-racial country with Malays, Chinese, and Indian as three major ethnic groups and each ethnic group commonly has its specific types of gene deletions.[7,8] -thalassemia carrier with 3.7 deletions was the most common type and was distributed evenly among the 3 ethnic groups followed by SEA deletion and then deletion of 4.2 kb of DNA. The 4.2 deletion type of α-thalassemia carrier was commonin Malay and SEA deletion was frequently detected among the Chinese.[9,10]

Therefore, the aims of this study are to detect α -globin gene deletions and evaluate the haematological parameters among deletional α thalassemia patients in Hospital Universiti Sains Malaysia, Northeastern Malaysia.

Materials and Methods:

Patient selection and sample collection

This cross-sectional study was carried out among 214 patients who attended Hospital Universiti Sains (HUSM) in Kubang Kerian, Kelantan, Malaysia and suspected for α thalassaemia in year 2014 until 2016. This study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia (USM/JEPeM/1701008). The full blood counts (FBCs) were carried out using the automated haematology analyser (Sysmex XE-5000™, USA). The Hb analysis was performed using cation exchange high performance liquid chromatography (CE-HPLC) (Bio-rad Variant II System, USA).Hb analysis chromatogram as shown in Ffigure 1.

Multiplex Gap-PCR

The genomic DNA was extracted from blood samples using a commercial kit (Macherey

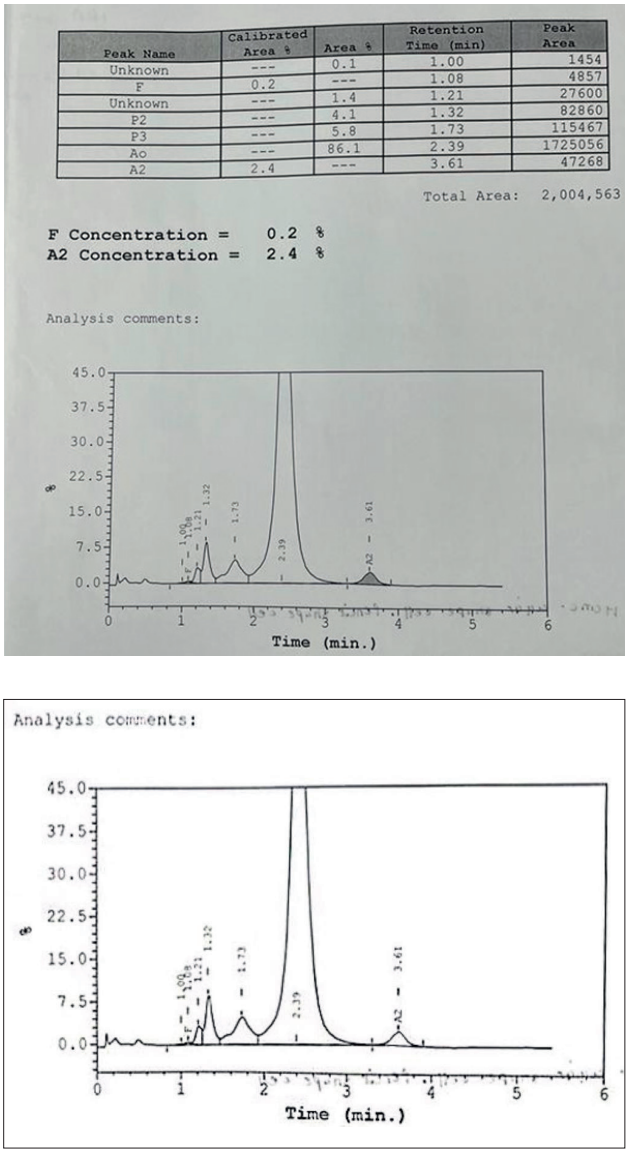

Figure 1: Hb analysis chromatogram

Nagel, Germany) according to the manufacturer's instructions. The extracted genomic DNA concentration was measured byusing Nanodrop ND-2000 Spectrophotometer (Thermo Fisher Scientific, USA). Multiplex Gap-PCR was performed for detection of [-α3.7, α4.2,--SEA]. The protocol for the PCR amplification was optimized and performed according to the multiplex PCR method described by Tan et al.[6]All amplifications were carried out using PCR machine Veriti 96 Well Thermal Cycler (Applied Biosystem, USA).The PCR products were run using a 1% agarose gel. The gel was visualized under ultraviolet (UV) light and theresultswere observed using AphaImager electronic imaging system (Alpha Innotech, USA) as shown in Ffigure 2.

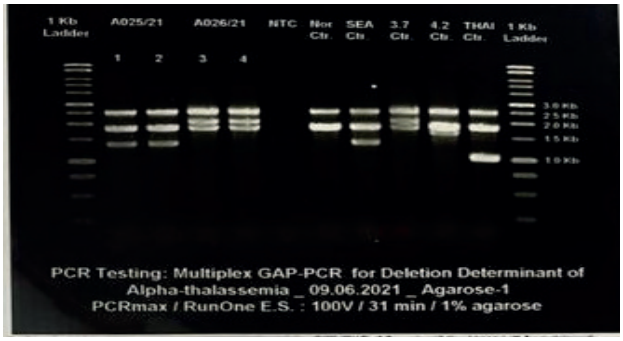

Figure 2: Gel picture for Multiplex Gap-PCR. (Abbreviation; Nor Ctr: Normal Control)

Statistical analysis

All statistical analyses were analyzed by using Statistical Package for the Social Sciences (SPSS) program version 22.0.

Result:

Out of 214 samples, 71 (46.7%) were detected for deletional type of α thalassaemia. SEA heterozygous was the most common type detected (50.7%). The frequency of deletional α thalassaemia was seen more in female subjects (61.7%) and more prevalent in the Malay (96.7%) (Table 1). Seven different genotypes of deletional α thalassaemia were detected in this study as shown in Table 21.

Table 1. Characteristics of samples profile, n = 214

Characteristics	Frequency, n (%)
Ethnic group	
Malay	207 (96.7)
Chinese	7 (3.3)
Gender	
Male	82 (38.3)
Female	132 (61.7)
Age	14.5 (26)*

*Median (IQR)

Table 2: Types of deletional α thalassaemia cases according to spectrum(n = 71)

Spectrum of deletional α thalassaemia	n (%)
SEA heterozygous	36 (50.7)
3.7 heterozygous	19 (26.8)
3.7 heterozygous with SEA heterozygous	7 (9.9)
4.2 heterozygous	5 (7.0)
3.7 homozygous	2 (2.8)
SEA with α variant	1 (1.4)
3.7 heterozygous with THAI heterozygous	1 (1.4)

There was a significant difference between the median of RBC, MCV and MCH level of patients with and without α deletion deletion (Table 3). Other haematological parameters including haemoglobin, RDW and platelet level showed no significant differences between two groups ($p > 0.05$).

Table 3: Haematological parameters comparison between sampel with and without α deletion deletion (n=214)

Variables	Molecular study for deletional α thalassaemia		Z statistic[a]	p-value
	Sample without deletional α thalassaemia Median (IQR) (n=143)	Sampel with deletional α thalassaemia Median (IQR) (n=71)		
Haemo-globin	11.2 (2.5)	11.3 (2.4)	-0.02	0.982
RBC	4.8 (0.6)	5.4 (0.6)	-6.07	< 0.001
MCV	70.8 (9.9)	65.4 (11.5)	-4.81	< 0.001
MCH	23.3 (4.4)	20.8 (3.5)	-5.09	< 0.001
RDW	16.2 (3.6)	15.9 (4.1)	-0.59	0.556
Platelet	318.0 (129.0)	326.0 (175.0)	-0.50	0.616

Mann-Whitney test was applied, $p<0.05$ as significant at 95% CI

There was a significant difference between the median of MCV, MCH, RDW and platelet level, between the 3 types α deletion in this study. However, no significant difference of the RBC value among the three groups as shown in Table 4.

Discussion:

Identification of α thalassemia trait by hematological parameters could significantly improve screening and prevention program in developing countries. The program could be conducted more efficiently by involving full range of staffing from the first line of medical health services until the research center.

In this study, 214 samples were sent for the investigation of α thalassemia because of hypochromic microcytic red blood cells with normal Hb A_2 and Hb F. 71 (33.2%) samples were detected with deletional α thalassaemia by molecular method. This result would represent the prevalence of deletional α thalassaemia in Kelantan. α thalassaemia are common in Malaysia and the neighbouring SEA countries. It was reported to be 30% in northern Thailand and 16% in southern Thailand[11], 2.6% - 3.2% of α^0 carriers and 2.7% - 11% of α^+ carriers in Indonesia[12].

There were 7 spectrums of α gene deletion detected

Table 4: The comparison of haematological parameters between the 3 types of deletional α thalassaemia, (n=62)

Parameter	Deletional α thalassaemia type			X^2statistic[a] (df)	p-value
	3.7 heterozygous (n=19)	SEA heterozygous (n=36)	3.7 heterozygous with SEA heterozygous (n=7)		
	Median (IQR)	Median (IQR)	Median (IQR)		
Haemoglobin	12.40 (2.50)	11.25 (1.50)	9.30 (0.80)	15.21 (2)	< 0.001
RBC	5.19 (0.87)	5.49 (0.57)	5.57 (0.50)	4.29 (2)	0.117
MCV	73.30 (11.10)	64.35 (10.50)	55.40 (10.40)	24.71 (2)	< 0.001
MCH	24.20 (3.50)	20.30 (2.40)	16.70 (2.4)	29.10 (2)	< 0.001
RDW	14.20 (2.40)	16.50 (3.40)	22.80 (2.80)	24.94 (2)	< 0.001
Platelet	282.00 (104.00)	350.00 (166.00)	400.00 (175.00)	7.05 (2)	0.029

[a] Kruskal-Wallis test, $p<0.05$ as significant at 95% CI

in this study. The commonest spectrum seen was two genes deletion, αα/--^{SEA} followed by 1 gene deletion, αα/-α$^{3.7}$ and 3 genes deletion --^{SEA}/-α$^{3.7}$. The proportion of one gene deletion (αα/-α$^{3.7}$ or αα/-α$^{4.2}$) in this study was 33.8%, 2 genes deletion (αα/--^{SEA} or -α$^{3.7}$/-α$^{3.7}$) was 53.5% and 3 genes deletion (--SEA/-α$^{3.7}$ or --^{THAI}/-α$^{3.7}$) was 11.3%. One case of presumed non-deletional HbH disease (αα^{CS}/--^{SEA}) was detected.

In Asian population, α^0 thalassaemia were commonly occur with the SEA deletion.[13] The SEA deletion α^0 thalassaemia was reported to be common in Chinese population with frequency of 48.54% in Guangdong Province[14] and 8.5% among the Chinese population in Malaysia. The 4.2 heterozygous deletions were frequently detected in Chinese patient with rate of 0.3%.[15] In this study, SEA deletion α thalassaemia was detected predominantly among Malay as the population in Kelantan is majority Malay.[16]

This study showed a significant difference between the median of MCH, MCV and RBC level in patients with and without α deletion. Therefore, RBC MCV and MCH count are an important parameter as a diagnostic adjunct in the screening for α thalassaemia. In α thalassaemia, the microcytic anaemia is associated with high RBC count compared to other causes of microcytic anaemia including iron deficiency and anaemia of chronic disease which are usually associated with low RBC count and proportionate with the degree of anaemia and also similar with this study.[4] In this study, the MCV level in α thalassaemia patient showed significantly lower value ranged from 54.5 fL to 77.9 fL. The typical classical finding of microcytic RBC has been known since decades. However, the cut off value of MCV to truly discriminate the other causes of microcytic RBC is difficult. All patients with microcytosis iron deficiency, any without chronic disease or do not respond to iron therapyshould proceed with thalassaemia screening.[17] A study by Lafferty et al suggested that a MCV of 72 fL is optimumly sensitive and specific for diagnosis of thalassaemia. By using MCV < 72.0 fL as a cut off value for thalassaemia testing, a sensitivity and specificity of 0.88 and 0.84, respectively was achieved regardless of the population.[17]A cut off MCV value < 80 fL was used in Hong Kong to detect all α^0 thalassaemia and most of α$^+$ thalassaemia.[18] This study was similar with previous reports, where microcytosis was correlate with the number of α-gene affected.[19]

This study found that MCH in deletional α thalassaemia group showed significantly low level with median ranged from 16.7pg to 24.2 pg. MCH is a better indicator compared to other red cell indices as it does not affect by age of the sample.[2] A study by Chan et al on archived samples from 255 school children found that MCH of < 27 pg could detect all α^0 thalassaemia effectively.[20] Other study also used MCH < 27 pg, MCV < 80 fL and HbA2 level (<3.5%) for a diagnosis of α thalassaemia trait.[21] This study found that all α thalassaemia subject would be effectively detected using MCH cut off value < 25 pg.

The haemoglobin concentration in the deletional α thalassaemia group showed marked heterogeneity ranged from normal to moderate anaemia. However, there was no significant difference in between deletional α thalassaemia group and no α gene deletion group were seen. Anaemia in α thalassaemia is correlated with the number of genes deleted.

The all 4 genes deletion α thalassaemia is not compatible with life producing a fetus with severe anaemia and hydropic, while 3 genes deletion α thalassaemia is associated with a moderately severe chronic haemolytic anaemia and mildly decreased haemoglobin concentration were seen in 2 genes deletion patients.[4] In Malaysia, anaemia is a common health problem. The prevalence of anaemia ranged from 35% - 57.4%.[22] A study by Al-Mekhlafi et al found that 48.5%of children with anaemia among the aborigine school children in Malaysia and 70.1% of them were due to iron deficiency anaemia.[23] Therefore, in area of high prevalence of both nutritional deficiencies and thalassemia, a comprehensive hematological parameter with genetic preventive program is important for the prevention of thalassemia (Dama & Dama., 2014).[24]

In this study, there were no significant median differences in between deletional α thalassaemia group and no α gene deletion group for RDW level. The RDW generally reflects the variable size distribution of the red blood cells.[6] The RDW was higher in iron deficiency anaemia patients reflecting the heterogeneous distribution of the erythrocytes compared to more homogeneous erythrocytes in thalassaemia patients.[25] However, other studies concluded that RDW could not be used as a single parameter to discriminate between iron deficiency and thalassaemia in cases of microcytic anaemia.[26] This showed that the value of RDW alone cannot be relied upon as a single screening parameter for thalassaemia but as adjunct with other haematological parameters. A study by Matos et al suggest that using RDW as a discriminatory marker between the microcytic anaemias is valid if the condition does not present with other disorders such anaemia of chronic disease.[26]

Conclusion:

This study highlighted the presence of different spectrums of α gene deletion in Kelantan population and the importance of molecular methods in establishing a definitive diagnosis of α gene deletion.

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Conflicts 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.

Authors's contribution

Data gathering and idea owner of this study: Rosnah B

Study design: Samilawati MA

Data gathering: Salman MS

Writing and submitting manuscript: Samilawati MA and Zefarina Z

Editing and approval of final draft: Marini R, Noor Haslina MN, and Shafini MY

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