ORIGINAL ARTICLE

Optimal Mean Corpuscular Haemoglobin (MCH) Cut-Off Value for Differentiating Alpha Plus and Alpha Zero Thalassaemia in Thalassaemia Screening

Faridah Idris[1], Chiu Yee Liew[2], Zainina Seman[1], Noraesah Mahmud[2]

[1] Department of Pathology, Faculty of Medicine and Health Science, University Putra Malaysia, 43400 UPM Serdang, Selangor
[2] Department of Pathology, Hospital Kuala Lumpur, 50586 Wilayah Persekutuan, Kuala Lumpur

ABSTRACT

Introduction: The aim of thalassaemia screening is to reduce thalassaemia syndromes with significant clinical implication. Therefore, detection of α^0 thalassaemia with two genes deletion is clinically more important than α^+ thalassaemia with one gene deletion. The aim of this study is to determine the mean corpuscular haemoglobin (MCH) cut-off point for α^0 thalassaemia screening. **Method:** A total of 688 α^0 and α^+ thalassaemia cases confirmed by DNA analysis were analysed. Red cell indices (MCV, MCH, RBC, Hb) were retrieved from the laboratory information system. Receiver operating characteristic (ROC) curve is generated to determine the MCH cut-off point for α^0 thalassaemia. The diagnostic performance of MCH cut-off value was evaluated with a validation group comprising 100 samples of alpha thalassaemia carriers. **Results:** ROC curve analysis with area under the curve (AUC) of 0.969 showed that MCH at cut-off of 23.5pg has high sensitivity and specificity in detecting α^0 thalassaemia with 98% sensitivity and 85% specificity. **Conclusion:** MCH cut-off value of 23.5pg can be adopted as the cut-off point for α^0 thalassaemia screening to detect clinically significant thalassaemia syndrome and reduce cost and burden of screening.

Keywords: α^+ thalassaemia, α^0 thalassaemia, Thalassaemia screening, MCH

Corresponding Author:
Faridah Idris, MPath
Email: i_faridah@upm.edu.my
Tel: +603-9769 2378

INTRODUCTION

Alpha (α) thalassaemia is the most common genetic disorder of haemoglobin synthesis affecting 5% of the world's population (1). It is very common in Southeast Asia and certain regions of China with approximately 40% of the population being carriers (2). In Malaysian population, single gene deletion, particularly the $-\alpha^{3.7}$ rightward deletion, is the most commonly seen in all ethnic groups except for Malaysian Chinese population (3). Among the Malays ethnic group, 4.3% of blood donors and 10.7% pregnant mothers carry this single gene deletion (4,5). Two genes deletion particularly $\_\ \_^{SEA}$ deletion, is more commonly seen in Malaysian Chinese population and this genotype is also the second most common α thalassaemia in other ethnic groups (3). Couples with alpha zero (α^0) thalassaemia which is characterized by cis positional loss of α gene (--/αα) are at higher risk of having foetus with Hb Bart's hydrops foetalis and this is consistent with the higher incidence found in Malaysian Chinese population compared to other ethnic groups in Malaysia (4).

α thalassaemia screening should focus on α0 thalassaemia detection to optimize the cost and avoid unnecessary anxiety among carriers with little clinical significance outcome. Full blood count (FBC) is an economical screening method because it is easily accessible for most health facilities, easy to operate and relatively cheaper compared to high performance liquid chromatography (HPLC) and electrophoresis. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) which are part of FBC parameters are low in thalassaemia (6) and are widely used in many countries for population screening (1). Both MCV and MCH are calculated parameters derived from automated blood analyser to indicate mean volume of red cell and the average amount of haemoglobin per red cell, respectively. However, other nutritional deficiencies such as iron, vitamin B12 and folic acid can affect MCH and MCV and make them unreliable in detecting thalassaemia carriers (6).

Malaysia has started national thalassaemia screening and registry for thalassaemia since 2004 (7) to reduce the

prevalence of transfusion dependent and other clinically significant thalassaemia syndromes. The screening used the value of MCH < 27pg as recommended in the BCSH guideline (8) as the cut-off point to do further tests such as high performance liquid chromatography (HPLC) or electrophoresis. If other thalassaemia, haemoglobinopathy and iron deficiency are excluded, molecular analysis is done to exclude α thalassaemia trait when the MCH is less than 25 pg. This is based on the few studies that showed a high percentage of α^0 thalassaemia carriers have MCH less than 25 pg (8, 9). However, the MCH level for alpha plus (α^+) carriers had a wide range of value and this may lead to false positive screening results and unnecessary molecular study (10, 11).

In this study we compared the MCH level in α+ and α0 thalassaemia carriers confirmed by the DNA analysis and subsequently determined the new MCH cut-off point for α0 thalassaemia as a potential decision-making aid in the screening program.

MATERIALS AND METHODS

This was a cross sectional study conducted in Haematology Laboratory, Hospital Kuala Lumpur (HKL). The data was taken from June 2015 to June 2017 and were referred from other centres throughout the country. 622 samples were required, calculated based on the percentage of exposure with outcome (Chinese ethnic with $--^{SEA}$ deletion mutation. 5.4%) and percentage of unexposed with outcome (other ethnic group with $--^{SEA}$ deletion, 1.1%) (4). The samples were selected by random selection from the list of molecular analysis results for α thalassaemia investigation as a sampling population.

Inclusion criteria included α+ and α0 thalassaemia confirmed by molecular analysis with normal HPLC findings. We excluded cases of compound α thalassaemia with other globin gene mutation, suspected concomitant iron deficiency (Hb less than 10g/dl) and incomplete data.

FBC, HPLC and molecular analysis results were retrieved from the laboratory information system. FBC results were generated from different haematology analysers from different centres. Sysmex XN 2000 was used in HKL for FBC measurement. Multiplex GAP PCR was used for the detection of seven common deletional mutations including single gene deletion ($-\alpha^{3.7}$, $-\alpha^{4.2}$) and two gene deletion ($--^{SEA}$, $--^{FIL}$, $--^{THAI}$, $--^{MED}$, $--(\alpha)^{20.5}$). Six common non-deletional mutations: initiation codon (ATG → A – G), codon 30 (ΔGAG), codon 35 (TCC → CCC) Hb Èvora, Codon 59 (GGC → GAC) Hb Adana, Codon 125 (CTG → CCG) Hb Quang Sze and termination codon (TAA → CAA) Hb Constant Spring were detected using Multiplex ARMS PCR test method.

Data were analysed by the Statistical Program for Social Sciences (SPSS) version 23. The demographic features of α thalassaemia carriers and distribution of different genotypes in α thalassaemia were analysed using descriptive tests. The difference of red cell indices (Hb, MCV, MCH, RBC) in α^0 and α^+ thalassaemia was analysed using t-test. The MCH cut-off point for α^0 thalassaemia was analysed using receiver operating characteristic (ROC) curve. The determined MCH cut-off point was evaluated with a validation group of 100 cases of α^0 and α^+ thalassaemia from another set of data. True and false positive together with true and false negative rates were determined and sensitivity and specificity in the validation group were obtained.

The study was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR-17-3198-38505) and the Ethics Committee for research involving Human Subjects, University Putra Malaysia (JKEUPM-2018-110).

RESULTS

A total of 688 α thalassaemia carriers data were recruited. Table I showed the demographic features of α thalassaemia carriers found in this study. Most were found to have α^+ thalassaemia (70.8%) and majority were females and from Malay ethnicity.

Table I : Demographic features

	α^+ thalassaemia	**α^0 thalassaemia**
	70.8%	**29.2%**
	(n=487)	**(n=201)**
Age (mean±SD)	22±11	24±12
Gender (n,%)		
Male	154 (31.6%)	76 (37.8%)
Female	333 (68.4%)	125 (62.2%)
Total	487 (100%)	201 (100%)
Ethnic (n, %)		
Malay	420 (86.2%)	96 (47.8%)
Chinese	25 (5.1%)	93 (46.3%)
Indian	19 (3.9%)	1 (0.5%)
Others	23 (4.7%)	11 (5.5%)
Total	487 (100%)	201 (100%)

Table II showed the distribution of different genotypes of α^+ and α^0 thalassaemia carriers in different ethnic groups. Majority of the α^+ thalassaemia carriers have deletional mutation (69.8%) and $-\alpha^{3.7}$ was found to be the most common genotype particularly in Malay ethnicity. For the non-deletional mutation, the majority of the subjects have termination codon (TAA → CAA) which is Hb

Constant Spring mutation. The most common genotype in α^0 thalassaemia was $--^{SEA}$ deletion and it was the most common genotype in Malaysian Chinese population.

Table II : Distribution of different genotypes in α thalassaemia carriers

	Malay (n, %)	Chinese (n, %)	Indian (n, %)	Others (n, %)	Total (n, %)
α^+ thalassaemia	**420**	**25**	**19**	**23**	**487**
(n=487)	(86.2%)	(5.1%)	(3.9%)	(4.7%)	(100%)
Deletional					
(n=340)	259	12	14	21	306 (90.0%)
αα/α-$^{3.7}$	(76.2%)	(3.5%)	(4.1%)	(6.2%)	
αα/α-$^{4.2}$	24	5	4	1	34
	(7.1%)	(1.5%)	(1.2%)	(0.3%)	(10%)
Total					**340**
					(100%)
Non-deletional					
(n=147)					
Initiation codon	0	0	0	0	0
αα/α^{Cd30}α	0	1 (0.7%)	0	0	1 (0.7%)
αα/α^{Cd35}α	0	0	0	0	0
αα/α^{Cd59}α	23 (15.6%)	0	0	0	23 (15.6%)
αα/α^{QZ}α	5 (3.4%)	3 (2.0%)	0	0	8 (5.4%)
αα/α^{CS}α	109 (74.1%)	4 (2.7%)	1 (0.7%)	1 (0.7%)	115 (78.2%)
Total					**147 (100%)**
α^0 thalassaemia					
(n=201)					
αα/$--^{SEA}$	87 (43.3%)	92 (45.8%)	1 (0.5%)	2 (1.0%)	182 (90.5%)
αα/$--^{FIL}$	6 (3.0%)	1 (0.5%)	0	9 (4.5%)	16 (8.0%)
αα/$--^{THAI}$	3 (1.5%)	0	0	0	3 (1.5%)
αα/$--^{MED}$	0	0	0	0	0
$-(\alpha)^{20.5}$	0	0	0	0	0
Total	96 (47.8%)	93 (46.3%)	1 (0.5%)	11 (5.5%)	201 (100%)

Red cell parameters analysed showed statistically significant difference in α^0 and α^+ thalassaemia ($p < 0.001$) as shown in Table III. Table IV showed the comparison of specificity and sensitivity of MCH cut-off value at 25pg and 23.5pg.

Table III : Comparison of MCV, MCH, Hb and RBC in α^+ and α^0 thalassaemia

	α^+	α^0	t statistic (df)*	P value	95% CI# of the difference
	Mean SD	Mean SD			
MCV (fl)	77.0 ± 4.5	66.7 ± 3.8	30.3 (435.7)	0.001	9.63 - 10.97
MCH (pg)	24.9 ± 1.7	20.7 ± 1.3	35.5 (486.4)	0.001	3.92 – 4.38
Hb (g/dl)	12.9 ± 1.4	12.2 ± 1.5	5.6 (686)	0.001	0.42 – 0.88
RBC (x 10^{12}/l)	5.2 ± 0.9	5.9 ± 0.7	-12.4 (289.2)	0.001	- 0.82 - - 0.62

* t statistic (degree of freedom)

Confidence interval

Table IV : Comparison of MCH cut-off value

MCH	Sensitivity	Specificity	AUC*	CI#	p value
≤ 23.5 pg	98%	85%	0.969	0.956 – 0.983	< 0.001
≥ 25.0 pg	99%	50%			

* Area under curve

Confidence interval

Based on the ROC curve (Fig.1), the best cut-off level of MCH level in predicting α^0 thalassaemia carriers is 23.5pg with the sensitivity of 98% and a specificity of 85% (95% CI: 0.956 – 0.983) with the area under the ROC curve (AUC) of 0.969.

When MCH cut-off point of 23.5pg was applied to the validation group (n=100), the positive predictive value was 78% and the negative predictive value was 98% with 97.5% sensitivity and 81.7% specificity. The findings are summarized in Table V.

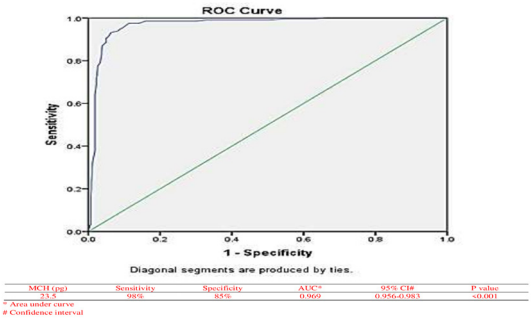

Fig. 1 : ROC curve of MCH in predicting α^0 thalassaemia. Based on the ROC curve (Fig.1), the best cut-off level of MCH level in predicting α^0 thalassaemia carriers is 23.5pg with the sensitivity of 98% and a specificity of 85% (95% CI: 0.956 – 0.983) with an area under the ROC curve (AUC) of 0.969.

Table V : Sensitivity and Specificity of MCH cut-off point of 23.5pg in the validation group.

	α^0 thalassaemia	α^+ thalassaemia
MCH ≤ 23.5 pg	78%	22%
MCH > 23.5 pg	2%	98%

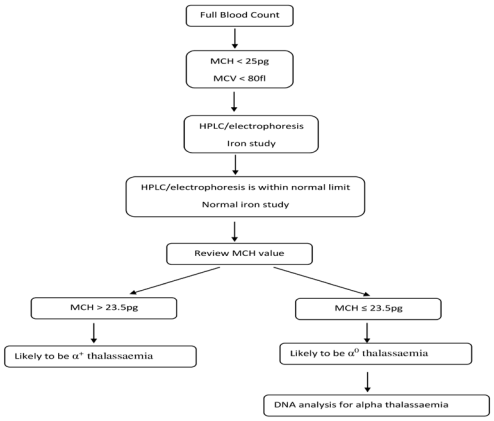

Fig. 2 : Schematic flow chart of alpha thalassaemia screening using MCH cut-off value of 23.5pg to detect α^0 thalassaemia.

DISCUSSION

The data showed the mean age of the patients with α^+ and α^0 thalassaemia was 22 and 24 years old, respectively. This is probably due to more premarital and antenatal screening as a result of increased awareness of the implications of thalassaemia carriers in offspring and consequently the cascade screening for families.

There is a slight female predominance of patients diagnosed to be thalassaemia carriers and may be attributed to more females being subjected for thalassaemia investigations during antenatal screening. According to a population-based anaemia screening in Malaysia, the prevalence of anaemia in females is significantly higher compared to male which were 34.50% and 14.4% respectively (12). Higher incidence of iron deficiency anaemia in females which have overlapping red cell parameters with thalassaemia carriers could be a reason for more cases being sent for DNA analysis.

The highest ethnic group of α thalassaemia carriers is Malay (75%) as it is the largest ethnic group in Malaysia and followed by Malaysian Chinese (17.2%) and other ethnic groups (4.9%) who are mainly subpopulation from Sabah and Sarawak. This finding is similar to most of the studies done in the local setting (3, 4, 13) and in concordance with the ethnic composition in Malaysia (14).

As α thalassaemia is protective against malaria infection, high frequency is seen in tropic and subtropic regions including Southeast Asia, Mediterranean, India subcontinent, Middle East and Africa (15). α^0thalassaemia commonly seen in Southeast Asia and Mediterranean predominantly $--^{SEA}$ and $--^{MED}$ respectively has a gene frequency of approximately 5% (15). $-\alpha^{3.7}$ deletion is the commonest α^+ thalassaemia with frequency of 70% and 90% reported in Melanesia and part of Nepal respectively (15). Among the 13 α thalassaemia genes determinants examined in this study, the most common gene mutation is $-\alpha^{3.7}$ rightward deletion followed by $--^{SEA}$ deletion and α^{CS} α mutation. The findings are in accordance with a few local studies (3, 4) and our neighbouring country Thailand (16). The α thalassaemia alleles found in Malaysian Chinese ethnic group, which showed the most common allele is $--^{SEA}$ followed by $-\alpha^{3.7}$ and $-\alpha^{4.2}$ is also similar to the analysis done in China (17). A study in Southern Taiwan also revealed that α thalassaemia is the most prevalent among thalassaemia and haemoglobinopathies and the most common genotypes were $--^{SEA}$ (69.4%) and Hb Quong Sze (1.54%) (18). Our findings added up to the growing data that the α gene allele frequency is different according to the geographical distribution and ethnic groups. This is important in deciding the approach in the thalassaemia screening program in a nation or region.

All the studied red cell parameters showed significant differences between α^+ and α^0 thalassaemia carriers and this is similar to the findings in most literatures (2, 3, 19). In most of the thalassaemia screening programmes, MCV and MCH are used as the parameters to identify individuals suspected to have thalassaemia and to proceed to further investigations. However, it is known that red cells swell in stored samples and may lead to falsely increased MCV level particularly if transportation and delay in sample processing is inevitable (6, 20). MCH is found to be more sensitive to screen for thalassaemia (20) and patients with MCH level less than 27pg will be investigated for thalassaemia (21) and by using this approach both α^+ and α^0 thalassaemia can be detected. However, as the main purpose of α thalassaemia screening is to detect clinically significant thalassaemia syndromes, detection of α^0 thalassaemia is more important than to detect α^+ thalassaemia.

In Malaysia, MCH cut-off of ≤ 27pg as an initial screening value is adopted as recommended in BCSH 2010 guideline for thalassaemia screening (7). In cases where iron deficiency and beta thalassaemia has been excluded, and when MCH is less than 25pg the samples are subjected for α thalassaemia DNA analysis mainly to identify carriers with two genes deletion as they are at risk to have offspring with HbH and hydrops feotalis if their partner is also an α thalassaemia carrier (7). Individuals with MCH 25-27pg will not be accepted for α thalassaemia DNA analysis unless there is positive family history or the partner is known to have α thalassaemia.

In this study, both α^+ and α^0 thalassaemia have MCH less than 25pg and this cut-off point has been shown not to be discriminatory between α^0 and α^+ thalassaemia. This fact is also comparable with few studies (3, 10, 22) that showed the mean MCH for both α^+ thalassaemia and α^0 thalassaemia was either lower than 25pg or were overlapped with each other.

Based on our data, MCH cut-off level of 23.5pg for identification of α^0 thalassaemia has higher specificity (85%) compared to the MCH cut-off level of 25pg (specificity of 50%) as used in the current national screening programme while maintaining good sensitivity of 98%. Our findings are also comparable with a previous study that showed MCH cut-off point at 23.40pg showed AUC 0.905 provides a sensitivity of 85.7% and a specificity of 78.50% (19). This slight difference of MCH value may be due to different populations and genetic alleles in the study. Exhaustive literature search related to MCH cut off value showed limited data on the MCH cut off value for α^0 thalassaemia alone but the available data showed MCH value for two-gene deletion were consistently less than 25pg (3, 10, 22).

In the validation group, when MCH cut-off value less than 23.5pg was applied, 78% of subjects were correctly classified into the α^0 thalassaemia and when MCH cut-off value more than 23.5 pg, 98% of cases were correctly classified as α^+ thalassaemia.

It resulted in 98.7% sensitivity and 81.7% specificity with 99% negative predictive value. With these encouraging figures, it is most likely to reduce the number of individuals needed for further α thalassaemia DNA analysis leading to more targeted screening program and subsequently reduces the cost and workload. At the same time, its high sensitivity (98%) will ensure α^0 thalassaemia subjects are not missed. The lower MCH cut-off value is more applicable for the national thalassaemia screening programme in secondary school students as the majority of the samples received in the laboratory are from this group. Although high prevalence of iron deficiency was seen among adolescents in certain regions in Malaysia, particularly in the rural and underprivileged areas, mean MCH level was found to be only at lower limit of normal value (27.1pg) rather than at a very low MCH level (23). Hence, by adopting MCH cut-off value of 23.5pg for α^0 thalassaemia, it is less likely to be affected by iron deficient status. One study in Thailand also showed that the major cause of anaemia among adolescents were thalassaemia and haemoglobinopathies rather than iron deficiency anaemia (24). Therefore, thalassaemia screening is important for this age group and by using this lower MCH cut-off point value, more effective screening program to detect α^0 thalassaemia can be adopted. Figure 2 is the suggested schematic flow chart of α thalassaemia screening using MCH cut-off value of 23.5pg to detect α^0 thalassaemia particularly for screening program in the secondary school. This flow chart can be incorporated into the more comprehensive flow chart of the national thalassaemia screening program (7).

Limitation in this study is the majority of the samples were sent for DNA analysis for investigation of microcytosis and only those with MCH less than 27pg were subjected for further investigation. Hence, the true incidence of different genotypes in α thalassaemia was not detected as some of the subtypes with normal haematological indices are missed by using the screening algorithm (2). Iron deficiency is another important cause of microcytic anaemia in our community and the iron status is unknown in the majority of individuals sent for α thalassaemia DNA analysis (23). Study showed that MCH of less than 24pg was sensitive in predicting iron deficiency anaemia in which this value overlapped with the cut off value for predicting α^0 thalassaemia in this study (25). The co-existence of thalassaemia and iron deficiency is also not uncommonly seen (23,26). Hence, the effect of iron deficiency on the haematological parameters cannot be totally excluded in this study.

CONCLUSION

Our study observed a high sensitivity and specificity of using MCH level less than 23.5pg as the cut-off point for α^0 thalassaemia screening. Implementation of the lower MCH cut-off value is expected to reduce the numbers of samples for DNA analysis and the workload in the laboratory but at the same time ensure individual with two genes defect are not missed in the screening.

However, further study needs to be carried out to ensure the validity of adopting new MCH cut-off value for national thalassaemia screening program. Cost-effectiveness analysis can also be performed to compare the relative costs and effectiveness of health outcomes of using different MCH cut-off points in α^0 thalassaemia screening.

ACKNOWLEDGEMENT

We would like to extend our gratitude to the specialists and laboratory staff of the Department of Pathology, Hospital Kuala Lumpur who had helped us to complete this study.

REFERENCES

1. Vichinsky E. Complexity of alpha thalassemia: growing health problems with new approaches to screening, diagnosis, and therapy. Ann N Y Acad Sci. 2010;1202(1):180-187.
2. Bain BJ. Haemoglobinopathy diagnosis. 2nd ed: John Wiley & Sons; 2008.
3. 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. Int J Mol Sci. 2016;14(9):18599-18614.
4. Aliza M, Nor Asiah M, Manaf A, Chin Y, Normi M. Prevalence and Disease Burden of Common Alpha Thalassemia Deletions in Malaysia n Blood Donors: A multi ethnic population. Int J Sci Res. 2012;2:1-5.
5. Wee YC, Tan KL, Chow TWP, Yap SF, Tan JAMA.

Heterogeneity in α-thalassemia interactions in Malays, Chinese and Indians in Malaysia. J Obstet Gynaecol Res 2005;31(6):540-546.
6. Trent RJ. Diagnosis of the haemoglobinopathies. Clin Biochem Rev. 2006;27(1):27-38.
7. Bahagian Pembangunan Kesihatan Keluarga. (BPKK). Modul Kaunseling Talasemia Di Peringkat Kesihatan Primer. 3rd ed: Kementerian Kesihatan Malaysia; 2016.
8. General Haematology Task Force of the British Committee for Standards in Haematology. The laboratory diagnosis of haemoglobinopathies. Br J Haematol. 1998;101:783-792.
9. Ryan K, Bain BJ, Worthington D, James J, Plews D, Mason A, et al. Significant haemoglobinopathies: guidelines for screening and diagnosis. Br J Haematol. 2010;149(1), 35-49.
10. Akhavan-Niaki H, Kamangari RY, Banihashemi A, Oskooei VK, Azizi M, Tamaddoni A, et al. Hematologic features of alpha thalassemia carriers. Int J Mol Cell Med. 2012;1(3):162.
11. Pharephan S, Sirivatanapa P, Makonkawkeyoon S, Tuntiwechapikul W, Makonkawkeyoon L. Prevalence of α-thalassaemia genotypes in pregnant women in northern Thailand. Indian J Med Res. 2016;143(3):315..
12. Awaluddin SM, Ahmad NA, Naidu BM, Mohamad MS, Yusof M, Razak MA, et al. A population-based anaemia screening using point-of-care in estimating prevalence of anaemia in Malaysia adults: findings from nationwide survey. J Community Med Health Educ. 2017;7(2):513.
13. Rahimah A, Nisha S, Safiah B, Roshida H, Punithawathy Y, Nurul H, et al. Distribution of alpha thalassaemia in 16 year old Malaysian students in Penang, Melaka and Sabah. Med J Malaysia, 2012;67(6):565-570.
14. Department of Statistics Malaysia. Population distribution and basic demographic characteristic report 2010; 2010. https://www.dosm.gov.my/v1/index.php?r=column/ctheme&menu_id=L0pheU43NWJwRWVSZklWdzQ4TlhUUT09
15. Piel FB, Weatherall DJ. The α-thalassemias. N Engl J Med. 2014;371(20):1908-1916.
16. Kulaphisit M, Kampuansai J, Leecharoenkiat K, Wathikthinnakon M, Kangwanpong D, Munkongdee T, et al. A comprehensive ethnic-based analysis of alpha thalassaemia allelle frequency in northern Thailand. Scientific reports. 2017;7(1):4690.
17. Lai K, Huang G, Su L, He Y. The prevalence of thalassemia in mainland China: evidence from epidemiological surveys. Scientific reports. 2017;7(1):920.
18. Wang HC, Hsieh LL, Liu YC, Hsiao HH, Lin SK, Tsai WC, et al. The epidemiologic transition of thalassemia and associated haemoglobinopathies in southern Taiwan. Ann Hematol. 2017;96(2):183-188.
19. Velasco-Rodríguez D, Blas C, Alonso-Domínguez JM, Vega G, Soto C, García-Raso A, et al. Cut-Off Values of Hematologic Parameters to Predict the Number of Alpha Genes Deleted in Subjects with Deletional Alpha Thalassemia. Int J Mol Sci. 2017;18(12):2707.
20. Karimi M, Rasekhi A. Efficiency of premarital screening of beta-thalassemia trait using MCH rather than MCV in the population of Fars Province, Iran. Haematologica. 2002;32(2):129-134.
21. Ryan K, Bain BJ, Worthington D, James J, Plews D, Mason A, et al. Significant haemoglobinopathies: guidelines for screening and diagnosis. Br J Haematol. 2010;149(1), 35-49.
22. Lin HJ, Shih MC, Peng CT, Liu TC, Chen KW, Shih HC, et al. Hematological features and molecular lesions of haemoglobin gene disorders in Taiwanese patients. Int J Lab Hematol. 2010;32(1p2):1-7.
23. Foo LH, Khor GL, Tee E-s, Dhanaraj P. Determinants of iron status in Malaysian adolescents from a rural community. Int J Food Sci Nutr. 2004;55(6):517-25
24. Pansuwan A, Fucharoen G, Fucharoen S, Himakhun B, Dangwiboon S. Anemia, iron deficiency and thalassemia among adolescents in Northeast Thailand: results from two independent surveys. Acta haematologica. 2011;125(4):186-92.
25. Alquaiz J, Abdulghani H, Khawaja R, Shaffi-Ahamed S. Accuracy of various iron parameters in the prediction of iron deficiency anemia among healthy women of child bearing age, Saudi Arabia. Iran Red Crescent Med J. 2012;14(7):397.
26. Lin CK, Chen LP, Chang HL, Sung YC. Underestimation of the coexistence of iron deficiencies and thalassaemia minors: a single institution experience in Taiwan. Kaohsiung J Med Sci. 2014;30(8):409-14.