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Prevalence of single and double thalassemia carriers in pregnant women and spouses: Case study of Sawanpracharak Hospital

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ABSTRACT

Background: Thalassemia is common in Thailand. Knowing the prevalence of thalassemia carriers in married couples would lead to proper management of this disease.

Objectives: This study was aimed to survey the prevalence of thalassemia carriers in pregnant women and spouses attending the antenatal care (ANC) unit at Sawanpracharak Hospital, Nakhorn Sawan Province, Thailand. Criterions to differentiate double thalassemia carriers and single thalassemia carriers were also aimed to propose.

Materials and methods: A retrospective study was conducted. Data of red cell parameters, hemoglobin typing, and globin gene genotyping were collected during 2012 to 2017. Study protocol was reviewed and approved by the Research Ethic Committee, Sawanpracharak Hospital.

Results: Both thalassemia diseases and carriers were found in the studied cohort. Prevalence of thalassemia carriers was 56.0% in which 7.6% found to be double thalassemia carriers of HbE/α thalassemia 1 (SEA and non-SEA type). HbE ($+A_2$), RBC count, MCV, MCH, and RDW were significantly different between the single HbE carriers and the double HbE/SEA-α thalassemia 1 carriers. Testing the previously established HbE, MCV, and MCH cutoff points demonstrated high efficiency in detecting this major double carrier.

Conclusion: Thalassemia and hemoglobinopathies were common in the married couples attending the ANC clinic at Sawanpracharak hospital. Both single and double thalassemia carriers were existed. The double HbE/SEA-α thalassemia 1 carrier was predominated and could be detected efficiently by the previously established cutoff points of HbE, MCV, and MCH.

Introduction

Thalassemia and hemoglobinopathies are inherited chronic anemia commonly found in Thailand. Genes of these disorders are inherited in an autosomal recessive fashion. Those having these genes in heterozygous or doubly heterozygous forms are called single and double thalassemia carriers, respectively. These carriers are in fact clinically asymptomatic. However, those having the genes for thalassemia and hemoglobinopathies in homozygote or compound heterozygote form have the clinical symptoms of thalassemia disease.[1] The most severe cases of thalassemia disease; *e.g*. Hb Bart's hydrops fetalis, always die before birth and just after birth and may induce toxemia of pregnancy in mother. In contrast, those severe thalassemia patients who are alive always require regular blood transfusion, and suffer from several clinical complications.

Prevalence of thalassemia carriers is high in Thailand, reaching the nationwide frequency of about 40%. The prevalence

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of the α-thalassemia carriers (both α-thalassemia 1 and α-thalassemia 2) is about 20-30%, while that of the β-thalassemia, HbE and Hb Constant Spring are 3-9%, 54%, and 8%, respectively.[2] Thus, it is highly possible that both single and double carriers exist in the thalassemia carriers encountered in Thailand.

Presence of double α/β-thalassemia carriers and double HbE/α-thalassemia carriers in samples initially diagnosed as the β-thalassemia carriers and HbE carriers has be noted in several studies. In Malaysia, prevalence of double α/β-thalassemia carriers were found to be 12.7%, in which 7.8% were the double carriers of the SEA-α thalassemia 1/β-thalassemia.[3] In China, 4.4% prevalence of double the SEA-α thalassemia 1/β-thalassemia was demonstrated.[4] In Thailand, survey in Lampang and Chiang Mai found that 6.4% of the β-thalassemia carriers and 15.2% of the HbE carriers had the co-existing α-thalassemia. Prevalence of the double SEA-α thalassemia 1/β-thalassemia carriers and double HbE/SEA-α thalassemia 1 carriers were found to be 5.2% and 11.2%, respectively, in this study.[5] Thus, it is highly possible for the double thalassemia carriers to exist in other parts of Thailand. Most importantly, survey of this kind has never been substantially conducted in the central part of Thailand.

Materials and methods

Study design

A retrospective study was performed in this research. A total of 382 laboratory records of married couples attending the antenatal care (ANC) unit, Sawanpracharak Hospital, during the years 2012-2017 were collected. These records included red blood cell parameters (red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW)), hemoglobin typing, SEA-α thalassemia 1 genotype. The protocol was initially reviewed and approved by the Research Ethic Committee, Swanpracharak Hospital (COE No. 02/2561).

Red blood cell parameters determination

Red blood cell parameters including RBC count (10^6/μL), Hb (g/dL), Hct (%), MCV (fL), MCH (pg) were determined by an automated blood cell analyzer (Sysmex XN-1000™ Hematology Analyzer, Kobe, Japan).

Hemoglobin (Hb) typing

Hb typing to determine the relative quantities of hemoglobin in blood was performed by both the cation-exchange high performance liquid chromatography (HPLC) (Beta-Thalassemia Short Program, Variant™Hb Testing System; Bio-Rad Laboratories, Hercules, California, USA) and capillary zone electrophoresis (CZE) (Capillarys 2 System; Sebia, EvryCedex, France). Normal Hb types of A_2A was seen in normal individuals, α-thalassemia carriers, and β-thalassemia carriers. Normal individuals and α-thalassemia carriers had HbA_2<3.5% (2.6±0.36% for normal, 2.3±0.47% for α-thalassemia 1 carriers), while the β-thalassemia carriers had HbA_2 from 3.5 to 10% (5.5±1.26%).[6] The HbE carriers had Hb types of AE (+A_2) by the cation exchange HPLC with the HbE (+A_2) levels of 27.8±7.50%.[6] In the CZE, Hb types of A, E, and A_2 were seen with HbE levels of 24.0±3.0% and HbA_2 levels of 4.0±0.4%.[7] However, HbE levels plus HbA_2 levels were reported in cases of CZE method.

SEA-α thalassemia 1 genotyping

The SEA deletion of α-thalassemia 1 (SEA-α thalassemia 1) was identified by modified Gap-PCR and relative quantitative PCR with dissociation curve analysis described elsewhere.[8,9] In the modified Gap-PCR, the amplified products sized 185 bp were specific for the SEA deletion, while those of 314 bp were specific for wild type allele. Thus, carriers of the SEA-α thalassemia 1 had two PCR products sized 185 bp and 314 bp. In contrast, normal individuals had only one PCR products sized 314 bp. In the relative quantitative PCR, the dissociation temperature (Td) of SEA-α thalassemia 1 was 86 °C, being the same for both heterozygote and homozygote. Heterozygote of the SEA-α thalassemia 1 was characterized, however, by calculating the threshold cycle (Ct) difference or delta Ct (ΔCt) values between the SEA-α thalassemia 1 gene and the albumin gene. The ΔCt values of normal, SEA-α thalassemia 1 heterozygote or carrier, and SEA-α thalassemia 1 homozygote were 11.99±2.88, 6.87±1.80 and 2.26±0.62, respectively.

Criterion of making the final diagnosis

RBC parameters, types and quantities of hemoglobin, and Gap-PCR results were taken together to establish the final diagnosis of thalassemia in all recorded subjects as shown in Table 1.

Table 1. Criterion for the final diagnosis of thalassemia and hemoglobinopathies used in the study.

Diagnosis	Hb (g/dL)/Hct (%)	MCV (fL)/MCH (pg)	Hb types	Gap-PCR for SEA-α thalassemia 1
Normal	≥12/≥36	≥80/≥27	A_2A, A_2≤3.5%	Negative
Single SEA-α thalassemia 1 carrier	Variable	<80/<27	A_2A, A_2≤3.5%	Positive
Single β-thalassemia carrier	Variable	<80/<27	A_2A, A_2:3.6-8%	Negative
Single HbE carrier	Variable	Variable	EA	Negative
Double HbE/SEA-α thalassemia 1 carriers	Variable	Variable	EA	Positive
HbE/β-thalassemia	Variable	Variable	EF	Negative
Double HbE/β-thalassemia with SEA-α thalassemia 1 carriers	Variable	Variable	EF	Positive
Homozygous HbE	Variable	Variable	EE	Negative

Table 1. Criterion for the final diagnosis of thalassemia and hemoglobinopathies used in the study. (continuous)

Diagnosis	Hb (g/dL)/Hct (%)	MCV (fL)/MCH (pg)	Hb types	Gap-PCR for SEA-α thalassemia 1
Double homozygous HbE and SEA-α thalassemia 1 carriers	Variable	Variable	EE	Positive
Double HbE/non SEA-α thalassemia 1 carriers	Variable	Variable	AE, E ≤20%	Negative
HbH disease	Variable	Variable	A_2AH	Positive
Non-SEA α thalassemia 1 carrier	Variable	<80/<27	A_2A, A_2≤3.5%	Negative
Hb Constant Spring homozygote	Variable	Variable	A_2ACS	Negative
AE Bart's disease	Variable	Variable	AE Bart's	Positive
HbE/HbKU	Variable	Variable	E with KU	Negative
Other abnormal hemoglobin	Variable	Variable	A2A with Abn Hb	Negative

Note. HbKU: Hb Korle-BU (β73:Asp->Asn)

Statistical analysis

Statistical analysis including descriptive statistics (mean, standard deviation (SD), standard error of the mean (SE), and inferential statistics (Student's t-test) were carried out using the statistical software. The p value of less than 0.05 was considered significantly different.

Results

Demographic data

Records of 382 married couples including 190 males aged 26.06±6.83 years old and 192 females aged 22.94±5.69 years old were studied. The subjects were 367 Thai (96.1%) and 15 Burmese (3.9%). For Thais, 345 (90.3%) were from the central Thailand, 13 (3.4%) were from the northern Thailand, 8 (2.1%) were from the northeastern Thailand, and 1 (0.3%) was from the southern part of Thailand. Majority of the cases were from Nakhon Sawan Province.

Prevalence of thalassemia carriers

Based on the results of Hb typing and the modified Gap-PCR, 66.2% of the subjects had thalassemia phenotypes (carriers and diseases), while 56.0% were carriers of α-thalassemia, β-thalassemia and HbE. Additionally, 7.6% of the subjects were found to be double carriers of HbE/α-thalassemia 1 (SEA and non SEA types). The rest double forms of thalassemia included double carriers of the HbE/β-thalassemia with SEA-α thalassemia 1, and of homozygous HbE and SEA-α thalassemia 1 (Table 2).

Table 2. Thalassemia phenotypes in 382 subjects.

Diagnosis	Thai No. (%)	Burmese No. (%)	Possible genotypes
Normal	119 (31.2)	11 (2.9)	β^A/β^A, αα/αα
Single SEA-α thalassemia 1 carrier	53 (13.9)		$^{--SEA}$/αα
Single β-thalassemia carrier	60 (15.7)	1 (0.3)	β^T/β^A
Single HbE carrier	67 (17.5)	1 (0.3)	β^E/β^A
Double HbE/SEA-α thalassemia 1 carriers	24 (6.3)		β^E/β^A, $^{--SEA}$/αα
HbE/β-thalassemia	8 (2.1)		β^E/β^T
Double HbE/β-thalassemia with SEA-α thalassemia 1 carriers	1 (0.3)		β^E/β^T, $^{--SEA}$/αα
Homozygous HbE	17 (4.4)	1 (0.3)	β^E/β^E
Double homozygous HbE and SEA-α thalassemia 1 carriers	2 (0.5)		β^E/β^E, $^{--SEA}$/αα
Double HbE/non SEA-α thalassemia 1 carriers	4 (1.0)	1 (0.3)	β^E/β^A, --/αα
HbH disease	5 (1.3)		$^{--SEA}$/-α
Non SEA-α thalassemia 1 carrier	3 (0.8)		--/αα
Hb Constant Spring homozygote	1 (0.3)		$\alpha^{CS}\alpha/\alpha^{CS}\alpha$
AEBart's disease	1 (0.3)		β^E/β^A, $^{--SEA}$/ -α
HbE/HbKU	1 (0.3)		β^E/β^{KU}
Other abnormal hemoglobin	1 (0.3)		β^{Abn}/β^A

Note. β^A: Normal β-globin chain, β^E: HbE globin chain, β^T: β-thalassemia globin chain, β^{KU}: Hb Korle-Bu globin chain (β73;Asp->Asn), β^{Abn}: Unidentified abnormal β-globin chain, α^{CS}: Hb Constant Spring globin chain

Hemoglobin pattern in single and double forms of thalassemia.

Hemoglobin pattern of A_2A was observed in both single β-thalassemia carrier and double β-thalasemia/SEA-α thalassemia 1 carriers. AE patterns were observed for both single HbE carrier and double HbE/SEA-α thalassemia 1 carriers. In addition, the EF pattern was observed in single HbE/β-thalassemia and double HbE/β-thalassemia with SEA-α thalassemia 1 carriers. Finally, the EE pattern was the seen in single homozygous HbE and double homozygous HbE/SEA-α thalassemia 1 carriers.

Although types of hemoglobin in blood of both single and double form of thalassemia were the same, the levels of these hemoglobins were found to be obviously different. HbE ($+A_2$) levels were lower in double HbE/SEA-αthalassemia 1 carriers than those of single HbE carrier. Levels of HbE and HbF were higher in the single HbE/β-thalassemia than those of double HbE/β-thalassemia with SEA-α thalassemia 1 carrier. In addition, the HbE levels were higher in double homozygous HbE/SEA-α thalassemia 1 carriers than those of the single homozygous HbE. Detail of this information is shown in Table 3 and Figure 1.

Table 3. Hb typing patterns in single and double forms of thalassemia. "a" and "b" indicate statistically significant difference (p<0.05), "c" indicates no difference (p>0.05).

Diagnosis	Hb types	HbA_2 level (%)	$HbE(+A_2)$ level (%)
Normal (128)	A_2A	2.9±2.2 [a, c]	
Single SEA-α thalassemia 1 carrier (53)	A_2A	2.8±2.1[a, c]	
Single β-thalassemia carrier (61)	A_2A	5.7±0.7[a]	
Single HbE carrier (68)	EA		26.1±3.5[b]
Double HbE/SEA-α thalassemia 1 carriers (24)	EA		20.3±2.9[b]
Single homozygous HbE (18)	EE		87.3±3.7[c]
Double homozygous HbE/SEA-α thalassemia 1 carriers (2)	EE		92.2±9.9[c]

Note: a and b: statistically significant difference (p<0.05), c: no difference (p>0.05)

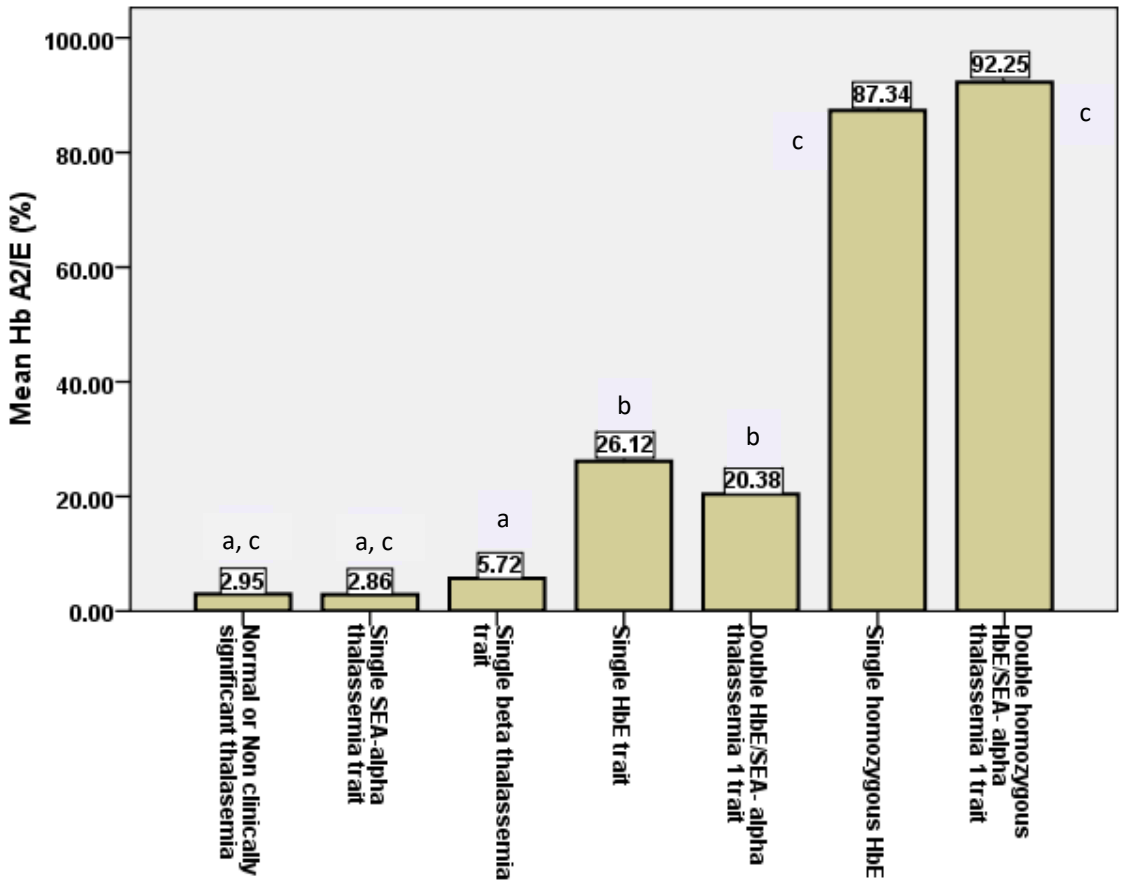

Figure 1 *Bar chart demonstrating the levels of Hbs A_2/E in single HbE carrier and double HbE/SEA-α thalassemia 1 carriers.*
Note: *a, b: significant difference within the tested groups (p<0.05), c: no difference in the pair tested (p>0.05). Mean values are placed at the top of each bar. Trait is a synonym for carrier.*

Red cell parameters of single and double form of thalassemia and hemoglobinopathies.

In HbE carrier group, RBC count, MCV, and MCH were significantly different between the single HbE carrier and double HbE/SEA-α thalassemia 1 carriers. RBC count was higher, while MCV and MCH were significantly lower in double HbE/SEA-α thalassemia 1 carriers than the single HbE carrier (Student's t test $p<0.05$) (Table 4, Figure 2).

In homozygous HbE group, 2 cases of double homozygous HbE/SEA-α thalassemia 1 carrier and homozygous HbE were encountered. RBC count, Hb, Hct, and MCV tended to be higher and RDW seemed to be lower in double homozygous HbE/SEA-α thalassemia 1 carrier than the single homozygous HbE. In contrast MCH were almost similar (Table 4, Figure 2).

Presence of the SEA-α thalassemia 1 carrier in the HbE/β-thalassemia seemed to reduce the hematological severity compared to the HbE/β-thalassemia without the SEA-α thalassemia 1 carrier. This was shown by higher levels of RBC count, Hb, Hct, and lower RDW in the HbE/β-thalassemia with co-existence of the SEA-α thalassemia 1 than in the HbE/β-thalassemia without the SEA-α thalassemia 1 trait.

Table 4. Red blood cell parameters (mean±SD) in the single and double form of thalassemia analyzed in this study.

Diagnosis	RBC count (10^6/uL)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	RDW (%)
Normal (105)	5.06±0.79	12.3±1.8	37.9±5.3	75.4±4.9	24.6±1.9	15.3±2.5
Single SEA-α thalassemia 1 carrier (49)	5.66±0.99	12.2±1.8	38.1±6.7	67.4±3.9	21.2±3.5	16.7±2.8
Single β-thalassemia carrier (52)	5.48±0.88	12.0±1.9	37.2±5.7	68.5±7.2	22.1±2.7	16.1±1.3
Single HbE carrier (56)	4.85±0.8	12.2±2.0	37.1±5.9	77.1±5.8	25.4±2.2	15.1±1.5
Double HbE/SEA-α thalassemia 1 carriers (24)	5.52±1.1	11.6±2.3	36.0±7.3	68.0±6.8	22.0±2.5	16.4±2.5
Single homozygous HbE (11)	5.50±1.00	11.8±1.7	34.8±7.3	64.8±4.8	20.7±1.5	16.8±1.5
Double homozygous HbE/SEA-α thalassemia 1 carriers (2)	5.78±0.58	12.0±1.5	37.8±5.5	65.3±3.1	20.7±0.6	16.1±0.1

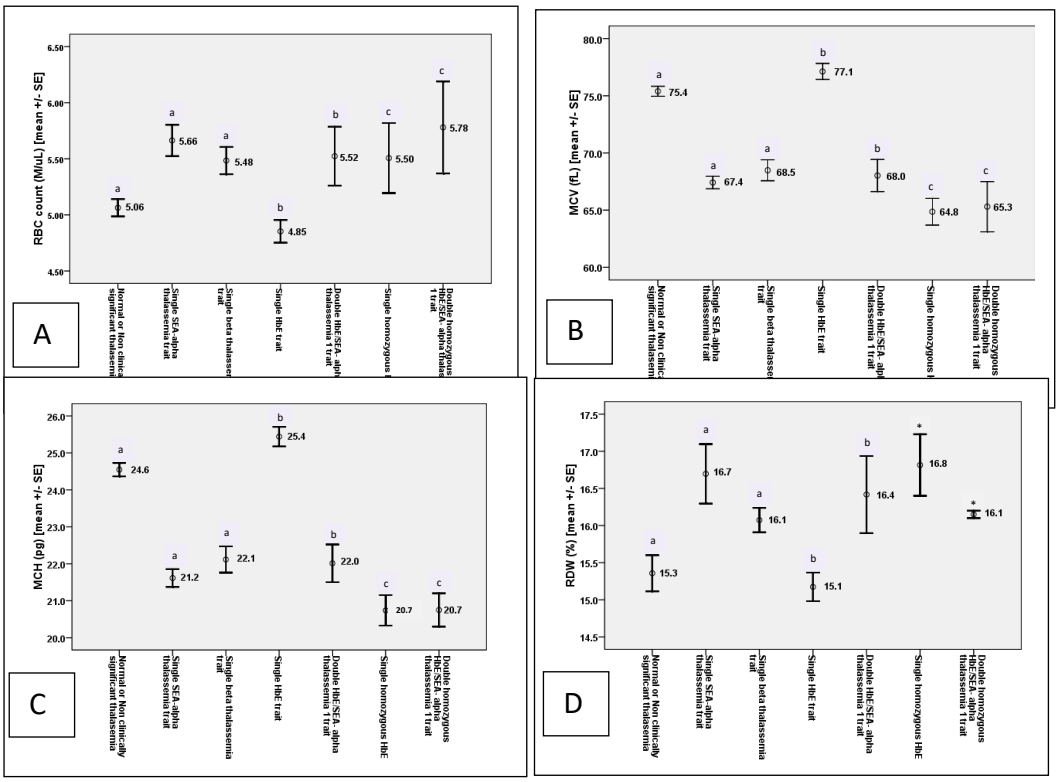

Figure 2 *Comparisons of RBC count (A), MCV (B), MCH (C), and RDW (D) between normal and single thalassemia carriers and double thalassemia carriers.*
Note: *a, b, *: significant difference within the tested groups (p<0.05), c: indicates no difference in the pair tested (p>0.05)*

Testing the previously established cutoff values to detect double HbE/SEA-α thalassemia 1 carriers.

As it was clear that the double HbE/SEA-α thalassemia 1 carriers predominated in this cohort, we then tested the previously established cutoff points in these 24 cases of this phenotype. These cutoff points relied on Hb levels. For Hb<10 g/dL, the cutoff points for HbE, MCV, MCH were 21.1%, 64.9 fL, 21.0 pg, respectively. For Hb 10-11.9g/dL, the cutoff points were 25.6%, 72.8%, 23.9 pg for HbE, MCV, MCH, respectively. Finally, for Hb≥12.0 g/dL, the cutoff points for HbE, MCV, MCH were 27.1%, 76.7 fL, 25.3 pg, respectively.[10] These cutoff points were found to have 94.7% sensitivity, 83.3% specificity, 64.3% positive predictive value (PPV), and 98.0% negative predictive value (NPV) in detecting the double HbE/SEA-α thalassemia 1 carriers in this cohort.

Discussion

This study has surveyed among the married couples for the prevalence of thalassemia in both carrier and disease forms. Majority of the subjects was from Nakhon Sawan and other provinces in the central Thailand. The high prevalence with the mixture of thalassemia found in this study should indicate that this disorder was also common in the central Thailand. Prevention and control scheme to detect the carriers of thalassemia must be implemented in order to prevent and control the emerge of new thalassemia patients.

Survey for thalassemia in antenatal care unit was performed previously in Mahararaj Nakorn Chiang Mai Hospital in which most of the subjects were from Chiang Mai Province.[11] This study found 25.6% prevalence of thalassemia (both carrier and disease) in their series. The finding in this study contrasted that in Chiang Mai survey by the higher prevalence of thalassemia and hemoglobinopathies. This emphasized the problem of this disorder in this geographic region, especially Nakhon Sawan Province since most of the subjects were of Nakhon Sawan origin.

Presence of double form of thalassemia and hemoglobinopathies were obvious in this study, being higher than that observed in Chiang Mai by Wanapirak, et al.[11] This information highlighted the problem of the double form of thalassemia in Nakhon Sawan Province and, possibly, other provinces in the central Thailand. Therefore, the laboratory personnel involved in thalassemia diagnosis must be greatly aware. Failure to detect double α/β-thalassemia carriers or HbE/SEA-α thalassemia 1 carriers can lead to the birth of the Hb Bart's hydrops fetalis babies which is not initially concerned.

The double α/β-thalassemia carriers and double HbE/α-thalassemia carriers have some laboratory results different from those in the single carriers. MCV, for instance, tends to be higher in double α/β-thalassemia carriers than the single β-thalassemia carriers.[12-14] However, these differences were not obvious. In contrast, both MCV, MCH, and HbE were shown previously to be significantly lower in double HbE/α-thalassemia 1 carriers than the single HbE carriers.[5,10,15,16] The result of the present study was identical to that observed in those studies. These similar findings indicated that MCV, MCH and HbE were the best parameters to be used to detect double HbE/SEA-α thalassemia 1carriers. Thus, we tested the cutoff points of MCV, MCH, and HbE as set up by Leckngam et al in this series of HbE carriers. The high sensitivity and moderate specificity demonstrated in this study indicated that MCV, MCH, HbE were suitable to screen out the double HbE/SEA-α thalassemia 1 carriers from the single HbE carriers.

In conclusion, this study clearly demonstrated the occurrence of variety of thalassemia as well as double thalassemia carriers in Sawanpracharak Hospital. The double HbE/SEA-α thalassemia 1 carriers predominated and could be simply screened by using MCV, MCH, and HbE, which are easily accessible for medical technologists. The information emerged from this study should alert clinicians, nurses, medical technologists, and other allied health personnel involved in thalassemia diagnosis in this part of Thailand to be aware of the existence of double thalassemia carriers, *i.e*. to prevent misdiagnosis of this fatal disorder.

Conflict of interest

None

Acknowledgements

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