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Case Report: Two cases of Hb Malay (HBB: c.59A>G) found in Northern Thailand

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ABSTRACT

Background: Hemoglobin (Hb) Malay is a common β-hemoglobinopathy in Malaysia resulting from an AAC to AGC mutation at codon 19, which produces an abnormal β-globin chain and manifests as a β^{+}-thalassemia phenotype characterized by mild anemia and increased HbA_2 levels. Although Hb Malay is commonly prevalent in Southern Thailand, there have been few reported cases in Northern Thailand.

Objectives: This study aims to report two cases of Hb Malay detected in Chiang Mai, Northern Thailand, highlighting the diagnostic complexities.

Materials and methods: Two female patients, aged 50 and 67, presenting with anemia were investigated. Initial hematological profiles, Hb analysis by HPLC (Case 1) and CE (Case 2), and iron studies were performed. Due to hypochromic-microcytic anemia and elevated HbA_2 levels in both cases, genomic DNA was extracted. Multiplex real-time PCR with HRM analysis was performed to detect common α^{0}-thalassemia deletions. Further genetic analysis was conducted using next-generation sequencing (NGS) targeting *HBA1, HBA2, and HBB* genes.

Results: Both cases were identified to carry the Hb Malay variant. Case 1, a 50-year-old female with mild anemia, was diagnosed with double heterozygosity for Hb Malay (β^{Malay}/β^{A}) and α^{+}-thalassemia ($-\alpha^{3.7}/\alpha\alpha$). Case 2, a 67-year-old female with severe anemia and iron deficiency, was diagnosed with heterozygosity for Hb Malay (β^{Malay}/β^{A}).

Conclusion: The diagnosis of Hb Malay can be complicated especially when it coexists with other thalassemia traits or iron deficiency. Therefore, better understanding of the hematological and clinical characteristics, as well as the laboratory detection of this hemoglobin variant, would be beneficial for genetic counseling, particularly in areas with a high prevalence of thalassemia, hemoglobinopathy, and iron deficiency such as Northern Thailand.

Introduction

β-thalassemia is a diverse group of inherited disorders prevalent in East Asian populations, characterized by point mutations or deletions affecting β-globin chain production. The two types are β^{0}-thalassemia, which involves the complete absence of β-globin chain production, and β^{+}-thalassemia, resulting in reduced synthesis. Numerous β-globin gene mutations have been identified across countries such as India, Thailand, and China.[1,2] In general, these mutations are population-specific, meaning each

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ethnic group exhibits its set of common mutations.

In Thailand, the prevalence of β-thalassemia ranges from 3% to 9%. Common β-globin gene mutations include codons 41/42(–CTTT) (HBB: c.126_129delCTTT) and codon17(A>T) (HBB: c.52A>T), which are observed nationwide. Additionally, HbE (HBB: c.79 G>A) is frequently found in Southern and Northeastern Thailand, while Hb Malay (HBB: c.59A>G) is more common in Southern Thailand.[3] Hb Malay is caused by an AAC to AGC mutation in codon 19, leading to Asn-Ser replacement and the production of an abnormal β-globin chain which first case was confirmed by DNA probe hybridization.[1,4] The mutation activates a cryptic RNA splice site in exon 1 of the β-globin gene, leading to abnormal RNA processing and β^+-thalassemia phenotype.[5]

Routine diagnostic methods such as dichlorophenol indophenol precipitation (DCIP) test, high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) are widely used to detect Hb variants. Screening for β-thalassemia typically relies on HbA_2 level. However, borderline HbA_2 samples should be performed DNA analysis to confirm carriers, as missed cases may increase the risk for at-risk couples having affected offspring.[6] Hb Malay is one of the commonest β-globin gene mutations among individuals with borderline HbA_2 (3.0-3.9%).[6] Moreover, it can co-elute or co-migrate with HbA on HPLC chromatogram and CE electrophoregram, complicating diagnosis.[1,4] Reverse-phase HPLC, peptide analysis, and DNA studies, and advanced molecular techniques such as Multiplex Amplification Refractory Mutation System (M-ARMS) PCR, allele-specific PCR, and reverse dot-blot allele-specific oligonucleotide (ASO) hybridization are used to detect Hb Malay.

Hb Malay has been reported as highly prevalent (2.0%) in Southern Thailand.[7] However, a previous study in Northern Thailand documented four cases of HbE-β^+-thalassemia (Hb Malay), of which only one case was clearly identified as co-inheritance with rare α^+-thalassemia mutation (cap +14 C>G in the α_2-globin promoter).[8] One of the present cases involves the co-inheritance of Hb Malay with the common α^+-thalassemia deletion ($-\alpha^{3.7}/\alpha\alpha$), a combination not previously reported in Northern Thailand. Therefore, this study reports two cases of Hb Malay detected in our thalassemia laboratory at the Associated Medical Sciences-Clinical Service Center (AMS-CSC), Chiang Mai University, Chiang Mai, Thailand. The findings expand the documented spectrum of Hb Malay-associated thalassemia in the Northern region, highlighting the necessity for comprehensive molecular testing to ensure accurate diagnosis and effective genetic counseling.

Case report

Case 1

A 50-year-old woman presented with mild anemia detected during a health screening. Her hematological profile showed Hb 11.4 gm/dL, mean corpuscular volume (MCV) 78.0 fL, and mean corpuscular hemoglobin (MCH) 25.9 pg. Hemoglobin analysis using HPLC (VARIANT β-thalassemia Short Program; Bio-Rad Laboratories, Hercules, CA, USA) revealed HbA 84.3%, HbA_2 5.4%, and HbF 0.8% (Figure 1A), which suggests that she has a β-thalassemia trait. Iron studies were within normal range: Ferritin 100 ng/mL, Serum iron 89 µg/dL, TIBC 283 µg/dL, and Transferrin saturation 31% (Table 1). These findings indicated mild microcytic-hypochromic anemia in the absence of iron deficiency.

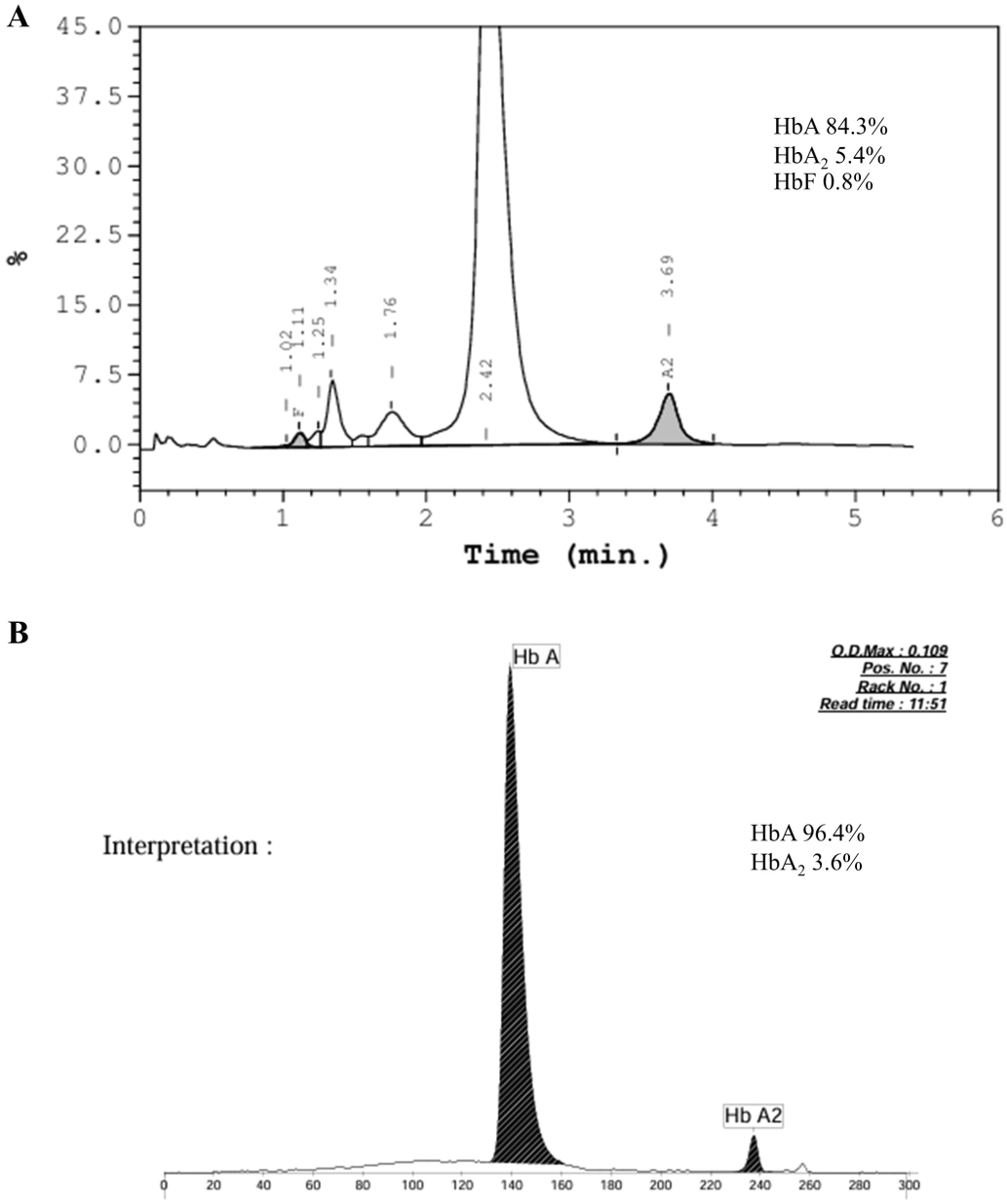

Figure 1. *The HPLC chromatogram (A) for Case 1, and CE electrophoregram (B) for Case 2, without seeing the separate peak of Hb Malay.*

Table 1. The characteristics and hematological parameters of cases.

Characteristics &hematological parameters	Case No. 1	Case No. 2
Age (years)	50	67
Gender	Female	Female
α-globin genotype	$-\alpha^{3.7}/\alpha\alpha$	αα/αα
β-globin genotype	β^{Malay}/β^{A}	β^{Malay}/β^{A}
RBCs ($x10^{12}$/L)	4.4	4.1
Total Hb (gm/dL)	11.4	6.4
PCV (L/L)	0.35	0.22
MCV (fL)	78.0	54.0
MCH (pg)	25.9	16.0
MCHC (gm/L)	325	290
RDW (%)	13.7	33.9
Ferritin (ng/mL)	100.0	12.1
Serum iron (mg/dL)	89.0	8.0
TIBC (mg/dL)	283	401
Transferrin saturation (%)	31.0	2.0
HbA (%)	84.3*	$96.4^{\#}$
HbA_2 (%)	5.4*	$3.6^{\#}$
HbF (%)	0.8*	$0.0^{\#}$

***Note**: Normal range of adults: red blood cell counts (RBCs) 4.2-6.1x10^{12}/L, total Hb 12.0-18.0 gm/dL, packed cell volume (PCV) 0.37-0.52 L/L, mean corpuscular volume (MCV) 80-100 fL, mean corpuscular Hb (MCH) 27.0-31.0 pg, mean corpuscular Hb concentration (MCHC) 320-360 gm/L, red cell distribution width (RDW) 11.0-16.0%, HbA 95.0-98.0%, HbA_2 1.5-3.5%, HbF 0.0-1.0%, ferritin 4.6-204.0 ng/mL, serum iron 50.0-170.0 mg/dL, total iron-binding capacity (TIBC) 259-388 mg/dL, transferrin saturation 20.0-50.0%. *Hb analysis by HPLC, #Hb analysis by CE.*

Case 2

A 67-year-old woman presented with severe anemia discovered during a routine laboratory screening. Her Hb was 6.4 gm/dL, with marked microcytosis (MCV 54.0 fL), low MCH (16.0 pg), and a red cell distribution width (RDW) of 33.9%. Iron studies showed severe deficiency results (Table 1). However, Hb analysis by CE (Capillarys 2 Flex piercing, Sebia, Evry, France) showed HbA 96.4% and elevated HbA_2 level (3.6%) (Figure 1B).

Due to the presence of microcytic-hypochromic anemia and elevated HbA_2 levels in both cases, genomic DNA was extracted from peripheral blood samples using the NucleoSpin® kit (Macherey-Nagel, KG., Duren, Germany) following the manufacturer's instructions. Subsequently, a single-tube multiplex real-time PCR with EvaGreen and high-resolution melting (HRM) analysis was performed to detect common α^0-thalassemia $--^{SEA}$, $--^{THAI}$, and $--^{CR}$ deletions, as previously described.[9] Neither patient carried these deletions. Further genetic analysis was performed using next-generation sequencing (NGS, BGI Group, Shenzhen, China), targeting the coding regions of the *HBA1*, *HBA2*, and *HBB* genes as previously described.[10] Both cases were found to carry the AAC to AGC mutation at codon 19 of the β-globin gene, leading to the Hb Malay variant (Figure 2). Furthermore, Case 1 had a single α-globin gene deletion ($-\alpha^{3.7}/\alpha\alpha$), whereas Case 2 did not. Thus, Case 1 was diagnosed as double heterozygosity for Hb Malay (β^{Malay}/β^{A}) and α^+-thalassemia ($-\alpha^{3.7}/\alpha\alpha$), while Case 2 was diagnosed with heterozygosity for Hb Malay (β^{Malay}/β^{A}) (Table 1).

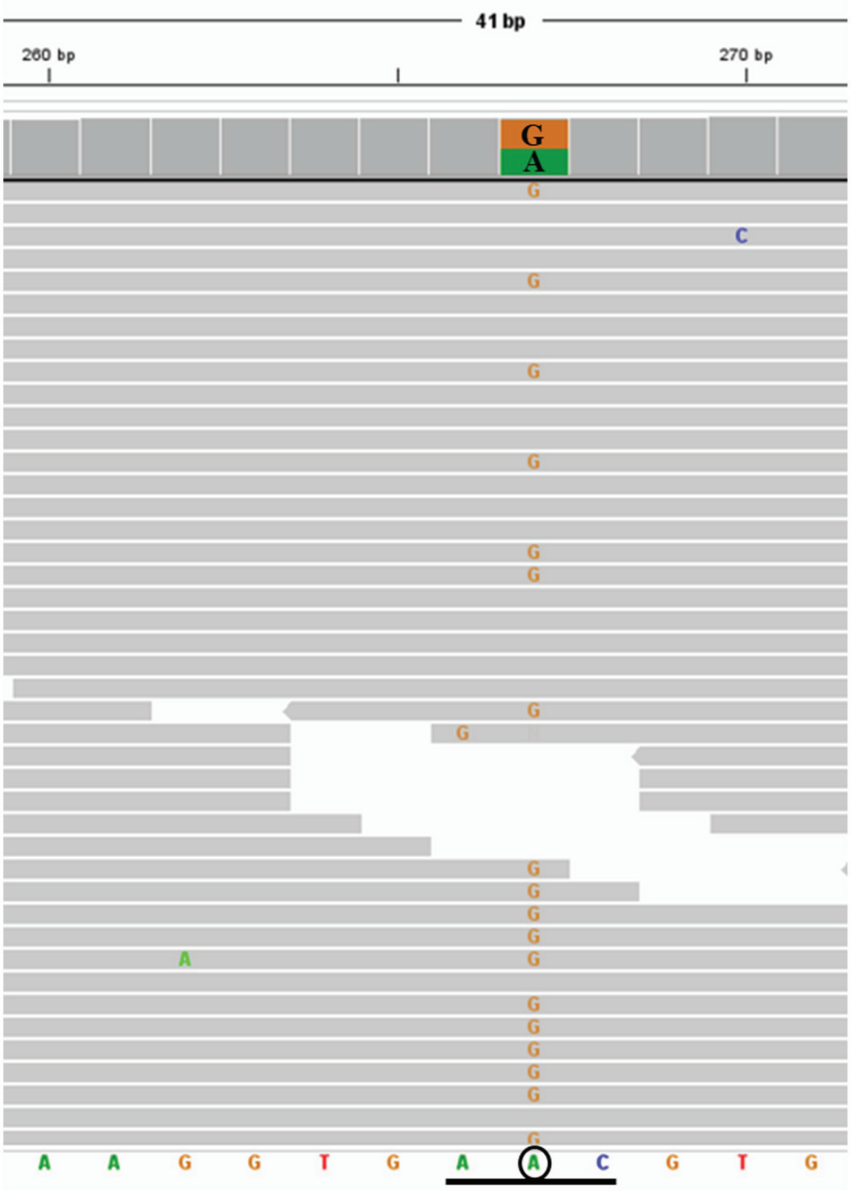

Figure 2. *Representative of NGS results exported from the integrative genomics viewer (IGV) of Hb Malay (HBB: c.59A>G).*

Discussion

Hemoglobin Malay was first reported in 1989 in a 22-year-old Malay patient.[4] The clinical features, hematological and biochemical findings in this patient and a sibling with homozygous Hb Malay showed mild to moderate hemolytic features consistent with thalassemia traits. Due to its HbA-like electrophoretic behavior with conventional electrophoresis, it was initially identified as β-thalassemia trait. However, unstable hemoglobin was detected in the patient and other family members, and a Hb variant eluting slightly later than the normal β-chains was seen by reverse-phase HPLC. The new variant was subsequently confirmed by hybridization of amplified DNA and later designated as Hb Malay.[1,4] In Thailand, hemoglobin variants are common and genetically heterogeneous among its population. Hb Malay is more prevalent in Southern Thailand and its detection in Northern cases may be related to historical migration or intermarriage with Southern populations. Even though an individual whose Hb typing appears normal or suggests a β-thalassemia trait with elevated HbA_2 (>3.5%), the presence of β-globin variants should be considered including Hb Malay.[6]

In Case 1, a 50-year-old female, her slightly low Hb, MCV and MCH values raised suspicion of thalassemia. Besides, her Hb analysis showed an elevated HbA_2 level (5.4%), suggestive of a β-thalassemia trait. The previous study reported that samples with double heterozygous for β- and α-thalassemia are usually diagnosed as typical β-thalassemia carriers and α-thalassemia is usually ignored.[11] However, there is no detectable common α^0-thalassemia $--^{SEA}$, $--^{THAI}$, and $--^{CR}$ deletions in Case 1. Nonetheless, subsequent molecular analysis,

conducted to identify other Hb variants, revealed double heterozygosity for Hb Malay and α⁺-thalassemia (-α$^{3.7}$). This case highlighted the possibility of misdiagnosis without further molecular study. In the previous study, the heterozygosity of Hb Malay without α-thalassemia co-inheritance showed MCV and MCH with 72 fL and 23 pg respectively. However, these values increase up to 75 fL and 25 pg in double heterozygosity of Hb Malay and α⁺-thalassemia,[7] which was consistent with our Case 1. This phenomenon could be due to the reducing the imbalance of α- and β-globin ratio.[12]

In contrast, Case 2 showed severe anemia with marked microcytic-hypochromic erythrocytes. Although her Hb analysis did not indicate any risk of thalassemia, her HbA_2 level was slightly increased, raising the possibility of β-thalassemia trait or a Hb variant. On the other hand, she was 67 years old, and her iron profile showed significantly deficient (Table 1) indicating iron deficiency anemia. Previous study has shown that iron deficiency anemia could reduce the level of HbA_2, potentially masking β-thalassemia trait.[13] Although initial hematological findings did not strongly indicate thalassemia, molecular analysis confirmed heterozygosity for Hb Malay. This case highlights the diagnostic complexity when iron deficiency coexists with hemoglobinopathies. Previous studies have reported that patients with heterozygosity for Hb Malay are typically characterized by mild anemia or normal Hb level in some cases.[1,14] However, Case 2 exhibited severe anemia (Hb 6.4 gm/dL). Thus, severe anemia in Case 2 was primarily due to iron deficiency anemia. Despite severe iron deficiency anemia, her red blood cell (RBC) count was nearly normal. This phenomenon may be attributed to the coexistence of β-thalassemia trait and iron deficiency anemia as previously reported.[15]

As Hb Malay exhibits HbA-like electrophoretic behavior, its diagnosis using conventional electrophoresis methods is challenging. Negligently interpreting such samples as normal could lead to at-risk couples giving birth to affected offspring. For accurate diagnosis of Hb Malay, techniques such as reverse phase HPLC, M-ARMS PCR, allele-specific PCR, and reverse dot-blot ASO hybridization can be applied. Furthermore, the NGS offers a comprehensive and robust diagnostic tool.

Conclusion

The diagnosis of Hb Malay can be complex, especially when accompanied by concurrent thalassemia characteristics or iron deficiency, resulting in misleading hematological profiles without molecular confirmation. Our findings highlight the importance of integrating hematological and molecular analyses for accurate diagnosis of this Hb variant, especially in regions where thalassemia, hemoglobinopathy and iron deficiency are prevalent such as Northern Thailand. Timely and accurate identification is essential for effective clinical management, prevention strategies, and genetic counseling.

Ethical approval

This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand (AMSEC-68EM-014).

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Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

CRediT authorship contribution statement

Moe Theingi: manuscript preparation; **Chedtapak Ruengdit:** conducting the experiments; **Manoo Punyamung:** conducting the experiments; **Sakorn Pornpraset**: designed and conducted all of the experiments, manuscript preparation. All authors have read and approved of the final manuscript.

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References

[1] Yang KG, Kutlar F, George E, Wilson JB, Kutlar A, Stoming TA, et al. Molecular characterization of β-globin gene mutations in Malay patients with Hb E-β-thalassemia and thalassemia major. Br J Haematol. 1989; 72(1): 73-80. doi: 10.1111/j.1365-2141.1989.tb07655.x.

[2] Leckngam P. Thalassemia and hemoglobinopathies in Thailand: A systematic review. J Health Sci Altern Med. 2023;5(3):104-13. doi: 10.14456/jhsam.2023.24.

[3] Paiboonsukwong K, Jopang Y, Winichagoon P, Fucharoen S. Thalassemia in Thailand. Hemoglobin. 2022; 46(1): 53-7. doi: 10.1080/03630269.2022.2025824.

[4] George E, Huisman TH, Yang KG, Kutlari F, Wilson JB, Kutlar A, et al. First observation of haemoglobin Malay alpha 2B2 26 (B1) Asn----Ser--a case report. Med J Malaysia. 1989; 44(3): 259-62.

[5] Yamsri S, Singha K, Prajantasen T, Taweenan W, Fucharoen G, Sanchaisuriya K, et al. A large cohort of β⁺-thalassemia in Thailand: Molecular, hematological and diagnostic considerations. Blood Cells Mol. Diseases. 2015; 54(2):164-9. doi: 10.1016/j.bcmd.2014.11.008.

[6] Bahar R, Shahida S N, Hassan mn, Marini R, mohd noor nh, Shafini M Y, et al. The diagnosis of beta thalassemia with borderline HbA2 level among

Kelantan population. J Blood Disord Transfus. 2017; 8: 5). doi: 10.4172/2155-9864.1000396.

[7] Tepakhan W, Kanjanaopas S, Sreworadechpisal K, Penglong T, Sripornsawan P, Wangchauy C, et al. Molecular epidemiology and hematological profiles of hemoglobin variants in southern Thailand. Sci Rep. 2024;14(1): 9255. doi: 10.1038/s41598-024-59987-4.

[8] Jomoui W, Satthakarn S, Panyasai S. Molecular understanding of unusual HbE-β(+)-thalassemia with Hb phenotype similar to HbE heterozygote: simple and rapid differentiation using HbE levels. Ann Med. 2023; 55(2): 2267054. doi: 10.1080/07853890.2023.2267054.

[9] Ruengdit C, Punyamung M, Intasai N, Pornprasert S. Single-tube multiplex real-time PCR with EvaGreen and high-resolution melting analysis for diagnosis of alpha0-thalassemia--^{SEA},--^{THAI}, and--^{CR} type deletions. PLoS One. 2023;18(11): e0293838. doi: 10.1371/journal.pone.0293838.

[10] Li R, Shen X, Chen H, Peng D, Wu R, Sun HY. Developmental validation of the MGIEasy Signature Identification Library Prep Kit, an all-in-one multiplex system for forensic applications. Int J Legal Med. 2021;135(3): 739-53. doi: 10.1007/s00414-021-02507-0.

[11] Al Hasan M, Nourin T. Co-inheritance of Alpha & Beta-Thalassemia: Case Report of a Bangladeshi Individual. Haematology Journal of Bangladesh. 2024; 8(1): 32-8. doi: 10.37545/haematoljbd2024117

[12] Winichagoon P, Fucharoen S, Chen P, Wasi P. Genetic Factors Affecting Clinical Severity in β-Thalassemia Syndromes. J Pediatr Hematol Oncol. 2000; 22(6): 573-80. doi: 10.1097/00043426-200011000-00026.

[13] Passarello C, Giambona A, Cannata M, Vinciguerra M, Renda D, Maggio A. Iron deficiency does not compromise the diagnosis of high HbA(2) β thalassemia trait. Haematologica. 2012; 97(3): 472-3. doi: 10.3324/haematol.2011.052357.

[14] Asri AS, Samsuddin MH, Jalil N, Mohamad Tahir N, Hashim H, Azma RZ, et al. Characterization of hemoglobin Malay phenotypes in tertiary hospitals. Hemoglobin. 2024; 48(3): 153-60. doi: 10.1080/03630269.2024.2380 873.

[15] Verma S, Gupta R, Kudesia M, Mathur A, Krishan G, Singh S. Coexisting iron deficiency anemia and beta thalassemia trait: effect of iron therapy on red cell parameters and hemoglobin subtypes. ISRN Hematol. 2014; 2014: 293216. doi: 10.1155/2014/293216.