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CASE REPORT

THE IMPORTANCE OF MOLECULAR BIOLOGICAL ANALYSIS FOR THE LABORATORY DIAGNOSTIC OF HOMOZYGOUS HAEMOGLOBIN MALAY

Bahar R[1,2], Zulkafli Z[1,2*], Zulkeflee RH[1,2], Hassan MN[1,2], Rahman Wan S Wan Ab[2,3], Noor NH M[1,2], Ramli M[1,2], Hussin A[1,2], Abdullah AD[1,2], Iberahim S[1,2], Abdullah M[1,2], Yusoff S M[1,2]

[*]**Corresponding Author:** Dr Zefarina Zulkafli, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan. Email address: zefarinazulkafli@gmail.com

ABSTRACT

Haemoglobin (Hb) Malay is variant haemoglobin with a β^{++} thalassemia phenotype. The prevalence of Hb Malay in the Malaysian population was 5.5%. We describe a 58-year-old male who presented with symptomatic anaemia to the Hospital Universiti Sains Malaysia. Further history revealed that the patient had anaemia since the age of 28, and on regular follow-up at other hospital. Physical examination revealed pallor, jaundice and hepatosplenomegaly. The full blood count and peripheral blood smear showed hypochromic microcytic anaemia with anisopoikilocytosis, and many target cells. High-performance liquid chromatography results showed a β thalassemia trait. However, the diagnosis does not alight with the patient's condition. Bone marrow aspirate was completed and showed reactive changes and erythroid hyperplasia. A molecular test was then performed for β globin gene mutation detection using Multiplex Amplification Refractory Mutation System (M-ARMS) PCR method. This revealed the result as homozygous codon 19 mutation or Hb Malay. Therefore, in this case report we would like to highlight the laboratory approaches, the challenges faced by the usual haematological investigations and the importance role of molecular testing in the diagnosis of severe anaemia.

Keywords: Hb Malay, thalassaemia, haemoglobinopathies, anaemia

[1] School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.

[2] Hospital USM, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.

[3] School of Dental Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.

INTRODUCTION

Almost 300 beta (β)-globin gene mutations have now been characterized (http://globin.cse.psu.edu). Some mutations completely inactivate the β gene, resulting in the absence of β-globin production that leads to β^0 thalassemia. Other types of mutations allow the production of some β globin and cause β^+- or β^{++} ("silent") thalassemia whereas β^{++} has more β globin production compared to β^+. β^0 / β^+ /β^{++} thalassemia phenotype depends on the site and nature of the mutation.[1] Therefore, the clinical and haematological spectrum of beta-thalassemia ranges from silent carrier to clinically manifested conditions, including severe transfusion dependent beta-thalassemia major and beta-thalassemia intermedia.[2]

The standard screening method for β-thalassemia includes full blood count (FBC), including the level of (MCV) < 80 fL and/or (MCH) <than 27 pg (being used as a cutoff level for a positive thalassemia screening result). The full blood picture (FBP) in thalassemia disease shows typical RBC morphology, consisting of microcytosis, hypochromia, and anisopoikilocytosis. Beta thalassemia major typically shows markedly elevated HbF (30-95%) levels with elevated HbA2. The proportion of HbA2 is dependent on the precise mutation of the β globin gene cluster.[3,4] Therefore, the minimal deficit of β-globin production is not associated with any consistent haematological changes and are the limitation of standard screening method for β-thalassemia in carriers of very mild-or-silent types of β-thalassemia.[3,4] Hence, the challenges faced during laboratory approaches and the importance of molecular genetic testing to confirm the diagnosis are discussed in this case report.

CASE REPORT

A 58-year-old male presented with symptomatic anaemia. Further history revealed that the patient has had

anaemia since the age of 28 and on regular transfusion and follow-up at other hospital. However, bleeding did not manifest. The patient has no history of trauma or fever. The patient also has no family history of haematological disorders. The physical examination revealed pallor, jaundice and hepatosplenomegaly (liver 13 cm and spleen 16 cm below costal margin) but no lymphadenopathy.

Laboratory results at presentation showed haemoglobin (Hb) 5.9 g/dL, total leukocyte count 6.43 X 10^3/μL, MCV 48.9 fl, MCH15.4 pg MCHC31.5 g/dL and platelet count350X 10^9/L. Other investigations, including coagulation profile (activated partial thromboplastin time, prothrombin time and fibrinogen), liver and renal function tests were all within normal ranges. The peripheral blood smear showed hypochromic microcytic anaemia with anisopoikilocytosis, and many target cells. Occasional nucleated red blood cell. No eosinophilia or basophilia (Figure 1).

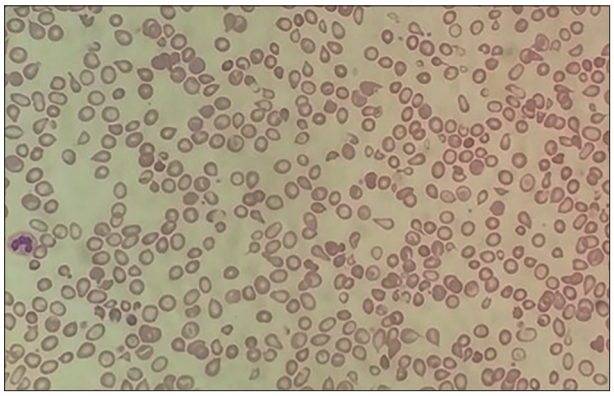

Figure 1. FBP stained with Wright Stain (X20 magnification)

Other investigations, including Hb analysis, high performance liquid chromatography (HPLC) showed A window (81.9%) with the presence of prominent peak at F (10.6%) and A2/E (7.5%) normal range is 2% to 3.2%. Alkaline gel electrophoresis showed prominent A2 band. The impression of Hb Analysis is a β thalassemia trait. The patient's bone marrow aspirate (BMA) showed reactive changes and erythroid hyperplasia. There was no evidence of acute leukaemia and other haematological malignancy. Later, Multiplex Amplification Refractory Mutation System (M-ARMS) PCR revealed homozygous codon 19 mutation/ Hb Malay.

DISCUSSION

Hb Malay was first described in 1989, being a β^{++} thalassemia phenotype with A → G mutation in codon 19, as detected in this case.[5,6] The prevalence of Hb Malay in the Malaysian population was 5.5%.[7] Homozygous Hb Malay usually presented with an average Hb of 7 to 8g/dL. Previously, it was reported that there was an increased production of Hb F between 9-25% in cases of homozygous Hb Malay and compound heterozygous Hb E/Malay.[6] This was also seen in this case, where the Hb F level in homozygous Hb Malay was 10.6%. Hb Malay (5.5%) was detected in northeast Thailand[8].

To date, many Hb variants have been discovered and can be detected by current screening methods for beta thalassemia; electrophoretic and HPLC methods. However, these techniques still have some limitations. It is because the available screening method is still unable to detect certain Hb variants with neutral substitutions.[6,7] It is difficult to diagnose a variant causing silent β-thalassemia, especially heterozygous Hb Malay because the haematological parameters and Hb A2 levels remain within a normal range. [9,10] Furthermore, as seen in this case, even though the Hb level is reduced, it is still challenging to confirm homozygous Hb Malay because both HPLC and capillary zone electrophoresis cannot differentiate between Hb A and Hb Malay. Hb Malay migrates as Hb A.[6-10] Therefore, the definitive diagnosis of Hb Malay can only be made via molecular analysis; M-ARMS PCR. Based on this case, the presence of a variant causing silent β-thalassemia should be considered and emphasized in unexplained clinical presentation typical of thalassemia.[6–9] Hence, it is a challenge or difficulty for the hospital or medical centre with no molecular technique facility to diagnose of Hb Malay. The hospital should therefore identify the nearest centre that has this service and send the sample to them for confirmation. Identification of this variant haemoglobin is important to prevent the birth of β-thalassemia major or intermedia children. Furthermore, for the couples at risk of conceiving a baby with β-thalassemia major or intermedia should be given genetic.[6]

CONCLUSION

In conclusion, well organized information, consisting of complete red cell indices and a Hb analysis result, together with a detailed history, including ethnic background, physical examination, and then followed up with molecular techniques such as M-ARMS PCR, can be used as a guideline for an effective tool in the investigation, detection, and confirmation of the diagnosis of Hb Malay. This is particularly important in the multi-ethnic populations of Malaysia as well as for proper clinical management of the patients.

REFERENCES

1. Thein SL. The molecular basis of β-thalassemia. Cold Spring Harb Perspect Med. 2013;3(5):1–24.
2. Traivaree C, Boonyawat B, Monsareenusorn C. Molecular analysis of beta-globin gene mutations among Thai beta-thalassemia children: results from a single center study. Appl Clin Genet. 2014;253.
3. Lee YK, Kim HJ, Lee K, Park SH, Song SH, Seong MW, et al. Recent progress in laboratory diagnosis of thalassemia and hemoglobinopathy: A study by the Korean Red Blood Cell Disorder Working Party of the Korean Society of Hematology. Blood Res. 2019;54(1):17–22.
4. Brancaleoni V, Di Pierro E, Motta I, Cappellini MD. Laboratory diagnosis of thalassemia. Int J Lab Hematol [Internet]. 2016 May 1 [cited 2022 Feb 20];38 Suppl 1:32–40. Available from: https://pubmed.ncbi.nlm.nih.gov/27183541/
5. Amran HS, Sarijan N, Sathar J, Md Noor S. Case series of homozygous and compound heterozygosity of Hb Malay, the diagnostic features and transfusion requirements. J Biomed Clin Sci [Internet]. 2017;3(2):10–7. Available from: http://apps.amdi.usm.my/journal/index.php/jbcs/article/view/180
6. George E, Huisman THJ, Yang KG, Kutlari F, Wilson JB, Kutlar A, et al. First observation of haemoglobin Malay. Med J Malaysia. 1989;44(3):259–62.
7. Yusoff YM, Hamid FS, Esa E, Aziz NA, Hassan S, Anoar SZ, et al. Molecular and Haematological Characterisation of Hb Malay in Malaysian Population. Asian J Med Biomed [Internet]. 2018 [cited 2022 Feb 20]; Available from: https://www.semanticscholar.org/paper/Molecular-and-Haematological-Characterisation-of-Hb-Yusoff-Hamid/5fd503a15bf5e5a039200a868e47b1a2c8b09fdf
8. Yamsri S, Singha K, Prajantasen T, Taweenan W, Fucharoen G, Sanchaisuriya K, Fucharoen S. A large cohort of β(+)-thalassemia in Thailand: molecular, hematological and diagnostic considerations. Blood Cells Mol Dis. 2015 Feb;54(2):164-9. doi:
9. Sharma S, Sehgal S, Das R, Gulati S. Phenotypic heterogeneity of delta-beta thalassemia. Indian J Pathol Microbiol [Internet]. 2019 Jan 1 [cited 2022 Feb 20];62(1):185–6. Available from: https://pubmed.ncbi.nlm.nih.gov/30706898/
10. Karim MUA, Moinuddin, Babar SU. Cap +1 mutation; an unsuspected cause of beta thalassaemia transmission in Pakistan. Turkish J Hematol. 2009;26(4):167–70.