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Submission date: 07-Apr-2023 03:36PM (UTC+0700)
Submission ID: 2058271463
File name: Compound heterozygous alpha-thalassemia 3.7 kb.pdf (371.24K)
Word count: 2566
Character count: 14161

CASE REPORT

Bali Medical Journal (*Bali MedJ*) 2021, Volume 10, Number 3: 1129-1132
P-ISSN.2089-1180, E-ISSN: 2302-2914

Compound heterozygous alpha-thalassemia 3.7 kb deletion and hemoglobin adana: a case report

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Received: 2021-08-08
Accepted: 2021-12-19
Published: 2021-12-30

ABSTRACT

Introduction: A point mutation of codon 59 (GGC → GAC) of the α2-globin gene, known as haemoglobin (Hb) Adana, contributes to various kinds of α-thalassemia syndrome. This case report described a patient who had heterozygous alpha-thalassemia 3.7 kb deletion and hemoglobin adana.

Case report: We report a case of a 17-year-old boy who was referred for the investigation of persistent anemia. His peripheral blood film was consistent with mild hemolytic anemia, without HbH inclusions. Normal HbA_2 levels without HbH and HbBart peaks. Both his parents had normal Hb levels, but his mother presented with mild microcytosis. DNA analysis revealed a compound heterozygote for one gene deletion ($\alpha^{-3.7}$) thalassemia and CD59 (GGC→GAC) mutation in the α2-globin gene. The mother was heterozygous for Hb Adana, and the father was heterozygous for $\alpha^{-3.7}$.

Conclusion: This case report emphasizes the need to consider Hb Adana detection in the absence of HbH inclusions and normal Hb analysis. Therefore, DNA analysis is strongly suggested to confirm the diagnosis and improve the management of thalassemia patients.

Keywords: HbH disease, alpha thalassemia mutations, Hb Adana, case.
Cite This Artide: Widyastiti, N.S., Limijadi, E.K.S., Retnoningrum, D., Nainggolan, I.M. 2021. Compound heterozygous alpha-thalassemia 3.7 kb deletion and hemoglobin adana: a case report. *Bali Medical Journal* 10(3): 1129-1132. DOI: 10.15562/bmj.v10i3.2629

INTRODUCTION

Alpha (⟨) thalassemia is the most common single-gene disorder. It is caused by the deletion or point mutation of the ⟨ globin gene. A non-deletional mutation usually leads to more severe disease compared to a deletional mutation. Non-functional defects that inactivate only one of the two alpha-globin genes are considered rare. Numerous variants of non-deletional mutations have been discovered, including Hb Adana.[1]

A point mutation causes hemoglobin Adana at codon (CD) 59 in alpha 1 or alpha 2 globin genes (GGC→GAC), which results in the transformation of glycine amino acid into aspartic acid (Gly→Asp).[2] Highly unstable hemoglobin variants, including Hb Adana, are troublesome to identify, unless the variant interacts with other types of alpha thalassemia, manifesting in Hemoglobin H (HbH) disease, a clinical condition that resembles beta-thalassemia intermedia.[3] The condition where a person experiences two different mutation variants at the alpha-globin gene cluster or two different mutation variants at the beta-globin gene cluster is known as compound heterozygosity.[1] A study conducted by Nainggolan et al.[4,5] among major and intermedia thalassemia patients found that the prevalence of hemoglobin Adana in Indonesia is relatively high but compound heterozygosity in alpha thalassemia and Hb Adana is rarely stated. Herein, we present a case of a compound heterozygous alpha-thalassemia genotype of 3.7 kb deletion with Hb Adana. Written informed consent was obtained from all the patients.

CASE REPORT

A 17-year-old male of Javanese ethnicity was referred to the clinical laboratory for the investigation of persistent anemia without a history of transfusion. The patient was given iron supplements and vitamins to improve his hemoglobin (Hb) level, but it remained below the reference range. Since childhood, every time the patient became feverish or infected, he turned pale and lethargic, with his eyes turning yellowish. The patient was the elder of two siblings. No family history of anemia or any other hematologic diseases was confirmed. Physical examination revealed splenomegaly of Schaffner 1. Analysis of the laboratory test results from the medical records revealed a history of low Hb levels, fluctuating mean corpuscular volumes (MCV) (from normocytic to mild microcytosis) within the preceding year, normal serum iron levels, normal levels of the G6PD enzyme, and unconjugated hyperbilirubinemia, with negative results of the antiglobulin test and HbH inclusion body test (Tables 1-3).

The peripheral blood film was consistent with the morphology of mild hemolytic anemia (Figure 1). Hb analysis using high-performance liquid chromatography (HPLC) revealed a normal level of HbA_2, and no HbH and HbBart peaks were detected (Table 3). The patient's father and siblings presented with normal hematological parameters, peripheral blood morphology, and Hb

analysis; however, mild microcytosis was detected in the mother (Table 2 and Figure 1). DNA analysis using multiplex PCR and PCR RFLP techniques confirmed the diagnosis of compound heterozygous α+ (α-3.7) deletion thalassemia and Hb Adana (point mutation at α2-globin gene CD59 (GGC→GAC)). The genetic inheritance of Hb Adana was discovered based on the family study and genetic analysis (Table 3).

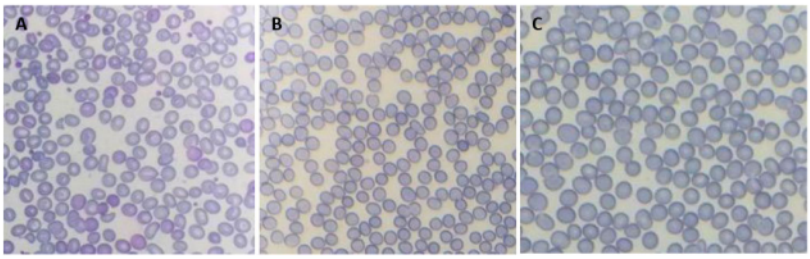

Figure 1. Photomicrograph of a peripheral blood film of the patient (A), his father (B), and his mother (C). The patient's blood film shows anisopoikilocytosis and the presence of target cells, teardrop cells, and a few fragmented RBCs. There was increased erythropoiesis as shown by the raised polychromatic cells and the presence of circulating nucleated RBCs. However, the blood films of the parents look normal and only that of the patient's mother shows mild microcytosis. (Giemsa stain, 400x).

Table 1. The patient's clinical chemical test results.

	Month 1	Month 12
SGOT/AST (U/L)	16	26
SGPT/ALT (U/L)	7	9
Gamma G (U/L)	14	
Total bilirubin (mg/dL)	**3.38**	**5.46**
Direct bilirubin (mg/dL)	**0.59**	**0.85**
Indirect bilirubin (mg/dL)	**2.79**	**4.61**
Albumin (g/dL)	**6.02**	
Globulin (g/dL)	2.02	
Total protein (g/dL)	8.04	
LDH (U/L)		296
Uric acid (mg/dL)		7.1
BUN (mg/dL)		29
Creatinine (mg/dL)		1.34
SI (ig/dL)	129	
TIBC (ig/dL)	253	
Saturation (ig/dL)	51	
Ferritin (ng/ml)	416.1	
HBsAg	Neg	
AFP (IU/ml)	< 0.50	
Direct Coomb test	Neg	
Indirect Coomb test	Neg	

The patient received vitamin E supplementation to prevent oxidative damage to the erythrocytes. He also received counselling about the potential genetic risks of having offspring with HbH disease or Hb Barts hydrops fetalis syndrome.

DISCUSSION

Thalassemia is the most common genetic disorder worldwide. It is due to the decrease or absence of synthesis or production of the globin chains that form hemoglobin.[1,2] Based on which globin chains have altered synthesis rates, thalassemia is genotypically categorized into thalassemia ⟨, ®, ©, ™, ™®, and ©™®.[1,6]

The main defect in alpha thalassemia is the reduced production of alpha-globin chains, which causes an imbalance in hemoglobin synthesis due to excess unpaired beta-globin chains. Unbound (unpaired) beta-globin chains precipitate in the red blood cell precursors within the bone marrow and peripheral blood, resulting in altered maturation of erythroid precursors, ineffective erythropoiesis, and shortening of the lifespan of the red blood cells.[1,7]

HbH disease resulting from a deletional mutation entails milder clinical symptoms and a possible need for intermittent transfusions, but the patients rarely need chronic transfusions. Meanwhile, in HbH disease caused by a non-deletional mutation, the patients may have splenomegaly and require regular transfusions.[1,3,7,8] In severe cases of alpha thalassemia (HbH disease), anemia develops from the ineffective erythropoiesis, which contributes to the shortening of the lifespan of red blood cells. These scenarios are the result of a complex combination of the harmful effect of beta-globin precipitation and the physical effect of HbH-containing cells having to pass through the microcirculation within the spleen.[1] Upon birth, a baby with HbH may present with specific hematological measures and have no remarkable symptoms. Clinical symptoms slowly develop in the first year of life, inciting the need for medical attention because laboratory test results are associated with hypochromic microcytic anemia or

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Table 2. The haematologic parameters of the patient and his family.

	Age (years)	Hb (g/dL)	RBC Count (10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Patient	17	9.1 (8.8 – 12.7)	3.68	79.8 (68.7-86.7)	24.7 (23.1 – 27.3)	31.0
Father	49	13.5	4.85	83.9	28.5	33.9
Mother	45	12.6	5.00	77.2	25.2	32.8
Sister	16	13.4	4.76	86.6	28.2	32.5

Table 3. The HPLC results and genetic test results of the patient and his family.

	HbA2 (%)	HbF (%)	Genetic test
Patient	2.4	1.7	Compound heterozygote of single α2- globin gene deletion and mutation in the α2-globin gene CD59 ($\alpha^{-3.7}/\alpha^{CD59,G\underline{G}C\rightarrow G\underline{A}C}\alpha$)
Father	2.8.	< 1	single α-globin gene deletion ($\alpha\,\alpha\,/\,\alpha\,\alpha^{-3.7kb}$)
Mother	2.8.	<1	mutation in the α2-globin gene CD59 (G<u>G</u>C→G<u>A</u>C)
SIster	2.9	<1	Normal

symptomatic anemia.[1,3,7]

Erythrocyte indices may vary among alpha thalassemia cases. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and Hb levels depend on the number of mutated genes and the extent of the reduction in α-globin chain synthesis following the mutations.[1,7] HbH disease due to a non-deletional allele mutation presents with lower Hb levels and higher MCV but a more severe phenotype compared to HbH disease due to a deletional mutation.[1] The HbH disease of this patient was caused by the interaction between a single gene deletional mutation (deletion 3.7 kb) and a non-deletional mutation (Hb Adana). Additionally, normal MCV might be influenced by erythroid hyperplasia which increases folate demand. Although the literature is scarce, there is a report of subclinical folate deficiency in HbH disease.[1]

Most HbH disease patients have normal iron levels or a slight increase in ferritin levels; however, they can have a significant increase in ferritin levels, especially if they are elderly patients, have regularly received blood transfusions, or have been receiving inappropriate iron therapy. A study by Weatherall et al.[1] found that there are no differences in the ferritin levels between HbH patients with deletional mutations and those with non-deletional mutations. Our case of compound heterozygous deletional and non-deletional alpha thalassemia presented with normal serum iron, thus corresponding to the study mentioned earlier.

The diagnosis of thalassemia can be challenging for clinicians. Presumptive laboratory diagnosis is based on the complete blood count (CBC) test results, erythrocyte morphology in blood smear preparation, HbH inclusion body test, iron status, and hemoglobin analysis using Hb electrophoresis or HPLC. Meanwhile, a definitive diagnosis of thalassemia requires molecular analysis.[9]

Both mild homozygous and heterozygous alpha-thalassemia with mild allele mutations should be diagnosed using molecular examination since routine laboratory tests often fail to identify alpha thalassemia. Laboratory test results of microcytic hypochromic anemia with normal iron status and normal HbA_2 levels raise the suspicion for alpha thalassemia. The patient in the present case report suffered from anemia since he was younger than five years; however, even though he frequently underwent medical check-ups, the diagnosis of hemoglobinopathy/thalassemia was difficult to establish by the clinician because of his fluctuating hemoglobin and MCV levels (from normal to slightly lower than normal) and both of his parents were healthy, with no symptoms. As described above, HbH disease patients with deletional and non-deletional type double mutations show varying hemoglobin and MCV levels, therefore, definitive diagnosis of this disorder requires molecular examination.

Although the prevalence of alpha thalassemia in Indonesia is relatively high, there have only been a few reports of compound heterozygous alpha-thalassemia and Hb Adana, possibly because most cases were underdiagnosed and unapparent during routine screening tests. The diagnosis remains challenging since Hb Adana is hyperunstable and cannot be visualized by routine screening methods. As shown in this case, the diagnosis of HbH would have been difficult if the clinical history, molecular analysis, and family study were not considered.

Family studies and genetic counselling are crucial in the management of thalassemia. Genetic counselling is a communication process that provides information and support to patients and their families about the diagnosis or the risk of genetic disorders. Genetic counselling in thalassemia aims to reduce or eliminate misconceptions about the causes of genetic diseases and to convey the correct information to increase the community's

control of their own health and their families by providing information about the diagnosis, treatment, and prevention of thalassemia.[10]

CONCLUSION

This case report emphasizes the importance of considering co-inheritance of the hyperunstable alpha Hb variant (Hb Adana) in α-thalassemia patients with negative HbH inclusion body test results and typical Hb analysis results. Family studies and DNA analysis are crucial to confirm the diagnosis and manage the persistent anemia caused by Hb variant/thalassemia.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest regarding publication of this article.

FUNDING

This report doesn't receive any specific grant from the government or any private sectors.

AUTHOR CONTRIBUTION

All authors had contributed for manuscript draft evaluation and writing, and agreed for the final version of publication.

ETHICAL STATEMENT

Patient and legal guardian had received signed written informed consent prior to data collection regarding publication of patient medical data in medical journals as a case report.

REFERENCES

1. Weatherall DJ, Clegg JB. The Thalassaemia Syndromes [Internet]. Blackwell Science Ltd; 2001. Available from: http://dx.doi.org/10.1002/9780470696705
2. Kalle Kwaifa I, Lai MI, Md Noor S. Non-deletional alpha thalassemia: a review. Orphanet J Rare Dis. 2020;15(1):166. Available from: https://pubmed.ncbi.nlm.nih.gov/32600445
3. Fucharoen S, Viprakasit V. Hb H disease: clinical course and disease modifiers. Hematology. 2009;2009(1):26–34. Available from: http://dx.doi.org/10.1182/asheducation-2009.1.26
4. Nainggolan IM, Harahap A, Ambarwati DD, Liliani R V, Megawati D, Swastika M, et al. Interaction of Hb adana (HBA2: c.179G>A) with deletional and nondeletional α(+)-thalassemia mutations: diverse hematological and clinical features. Hemoglobin. 2013;37(3):297–305. Available from: https://pubmed.ncbi.nlm.nih.gov/23614625
5. Nainggolan IM, Harahap A, Setianingsih I. Hydrops fetalis associated with homozygosity for Hb Adana [alpha59(E8)Gly-->Asp (alpha2)]. Hemoglobin. 2010;34(4):394–401. Available from: https://pubmed.ncbi.nlm.nih.gov/20642338
6. Bain BJ. Haemoglobinopathy Diagnosis [Internet]. Wiley; 2020. Available from: http://dx.doi.org/10.1002/9781119579977
7. Harteveld CL, Higgs DR. Alpha-thalassaemia. Orphanet J Rare Dis. 2010;5:13. Available from: https://pubmed.ncbi.nlm.nih.gov/20507641
8. Chui DHK, Fucharoen S, Chan V. Hemoglobin H disease: not necessarily a benign disorder. Blood. 2003;101(3):791–800. Available from: http://dx.doi.org/10.1182/blood-2002-07-1975
9. Clarke GM, Higgins TN. Laboratory Investigation of Hemoglobinopathies and Thalassemias: Review and Update. Clin Chem. 2000;46(8):1284–90. Available from: http://dx.doi.org/10.1093/clinchem/46.8.1284
10. Cao A, Kan YW. The prevention of thalassemia. Cold Spring Harb Perspect Med. 2013;3(2):a011775–a011775. Available from: https://pubmed.ncbi.nlm.nih.gov/23378598

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