Case Report

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A rare hemoglobinopathy duo: Hb Adana × Hb SEA in a 1-year-old patient – a case report and a brief literature review

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Introduction and importance: Alpha thalassemia, resulting from nondeletional mutations, typically presents a more severe clinical manifestation compared to deletional mutations. Severe outcomes, such as hydrops fetalis, are associated with two specific nondeletional mutations. Therefore, DNA-based investigation is crucial for suspected carriers exhibiting subtle hematological abnormalities to facilitate proper diagnosis and effective family counseling.

Case presentation: In this report, the authors describe a phenotypically normal 1-year-old girl with a rare and unique alpha-thalassemia genotype due to the presence of Hb Adana, a nondeletional alpha-chain mutation compounded with Hb SEA, an alpha-globin gene deletion.

Clinical discussion: Mutations determine the clinical manifestations of alpha-thalassemia. DNA testing is recommended for suspected carriers with relatively small hematological abnormalities, for precise diagnosis and family counseling. To provide clinicians with a reference for diagnostic assessment, the authors established a genotype-phenotype correlations based on reported cases of Hb Adana following an exhaustive literature review. Being interested in determining which ethnicities and genotypes are associated with a higher risk of complications, including hydrops fetalis and transfusion dependence, the authors formalized a diagnostic evaluation guide and a guide for early screening to improve outcomes.

Conclusion: Precise genetic evaluation is important for the diagnosis of alpha thalassemia. Hematologists play a critical role in managing these disorders, understanding genotype-phenotype correlations, and highlighting the significance of genetic counseling for high-risk patients. Extensive studies on these various genophenotypes are required to improve the diagnosis and prognosis of such medical conditions and advocate preventative strategies.

Keywords: alpha thalassemia, genotype-phonotype, Hb Adana, Hb SEA, hemoglobinopathy, mutations

Introduction

Alpha thalassemia arises from inherited mutations in alpha globin genes, leading to a reduction or absence of alpha globin production, or the presence of abnormal alpha globin structures that are crucial for stable hemoglobin (Hb) tetramers. Similar to other frequent globin gene disorders, α-thalassemia is highly prevalent in all tropical and subtropical locations worldwide. In certain regions, 80–90% of people carry the α-thalassemia gene, nearly to the point of fixation[1–5]. This prevalent hereditary condition affects ~5% of the global population, with some regions, particularly Southeast Asia, experiencing a high frequency of mutations[6–9]. In Southeast Asia, the prevalence of α-thalassemia is 22.6%. The countries with the highest frequencies of α-thalassemia were Vietnam (51.5%), Laos (26.8%), Thailand (20.1%), Cambodia (39.5%), and Malaysia (17.3%)[10].

The number of mutated α-globin genes is directly correlated with the phenotype of α-thalassemia. They are classified as α0-thalassemias, where both α genes are deleted (–/αα), and α+-thalassemias, when one of the connected pairs of α genes is removed (–α/αα). While the heterozygous state of α0-thalassemia results in the α-thalassemia trait, which is hematologically comparable to the β-thalassemia trait, the heterozygous state of

HIGHLIGHTS

- A rare form of alpha thalassemia in a 1-year-old baby who had hemoglobin Adana combined with hemoglobin SEA was reported.
- How it manifests clinically is conditional on the number and kind of defective alpha-globin genes.
- DNA-based investigation is crucial for suspected carriers to facilitate effective family counseling.
- Hematologists play an important role in assuring precise diagnosis and genetic counseling in high-risk individuals.

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α+-thalassemia is clinically silent. A similar phenotype was produced by the α+-thalassemia homozygous condition. Hb H illness is caused by the inheritance of both α+-thalassemia and α0-thalassemia (–α/–). Furthermore, α-thalassemia can also manifest in nondeletional variants (–αND/αα), which result from different structural anomalies in an α-globin gene. Hb H has a more severe form when it is inherited along with α0-thalassemia (–αND/–). Because Hb H is an unstable tetramer of β-chains (β4) with an overly strong affinity for oxygen, it cannot efficiently carry oxygen to cells. Ineffective erythropoiesis and hemolytic anemia are also caused by this somewhat unstable condition[11]. Chronic anemia is more severe in Hb H–Constant Spring than in deletional Hb H disease and often results in transfusion therapy in childhood[12].

Patient identification was aided by red cell and Hb indices. However, these are general results. It is possible to observe the H band by Hb electrophoresis. Molecular genetic tests are necessary for a definitive diagnosis[11]. Rapid diagnosis of the majority of deletional and nondeletional mutations is possible with multiplex genotyping tests employing gap polymerase chain reaction[13].

Diagnostic measurements include complete blood count, which can be performed in the laboratory using automated hematology analyzers. Nevertheless, Hb, mean corpuscular volume (MCV), and mean corpuscular Hb concentration (MCHC) were unable to distinguish between α-thalassemia and β-thalassemia or between thalassemia trait and iron deficiency[14]. Raised red blood cell counts are a characteristic of thalassemia that can help distinguish it from iron deficiency anemia, which is characterized by a low red blood cell count[15]. Target cells, teardrop cells, basophilic stippling cells, and microcytic hypochromic anemia were all observed in peripheral smear investigations. These results are consistent with those of iron-deficient anemia. HbH illness is indicated by the presence of 'golf ball-like Hb inclusions'.[14]. It is possible to quantify various forms of Hb in a patient's blood using HPLC or electrophoresis methods. These methods can be used to identify thalassemia disease types or carriers. These procedures also exhibit high accuracy[16]. DNA sequencing, real-time PCR with melting curve analysis, gap-PCR, reverse dot blot analysis, and allele-specific PCR can be used to identify mutations. Primer sequences have been published to diagnose multiple α+ or α° deletional mutations[17,18].

Patients with Hb H need to have their growth, quality of life, and test markers of organ dysfunction continuously monitored. Dietary supplements containing folate and multivitamins without iron[12]. Patients may become deficient in calcium and vitamin D. It is recommended that older people monitor their bone density. Therefore, it is best to refrain from using iron supplements. Making the choice to transfuse patients over the long term is challenging. Transfusions may be indicated for brief periods of time throughout pregnancy and childhood development[12,19,20].

This case report and brief review discuss a case of a 1-year-old girl, where DNA analysis revealed a heterozygous SEA α-gene deletion and her father's heterozygosity for a rare nondeletional alpha-thalassemia mutation at codon 59 (GGC → GAC), identified as Hb Adana[3–5]. To address diagnostic complexity, we conducted a literature review encompassing all reported cases of Hb Adana and Hb SEA. The objective of this review was to establish genotype-phenotype correlations, providing valuable prognostic information for families affected by these mutations in an effort to improve outcomes by developing a strategy for discovering such cases and boosting preventative screening programs.

Case presentation

We present a compelling case involving a 1-year-old female infant from the Philippines who was grappling with a rare form of alpha thalassemia. The intricate medical journey begins with a 33-year-old mother who had previously experienced several pregnancies all tragically culminating in neonatal death attributed to unexplained hydrops fetalis.

During the latest pregnancy, the mother underwent intrauterine blood transfusions at 21 and 25 weeks of gestation, which were prompted by the recurrence of hydrops fetalis detected on ultrasonography. The baby was delivered at 35 weeks' gestation and has been undergoing monthly blood transfusions since then. DNA analysis revealed that the mother carried a heterozygous SEA α-gene deletion, whereas the husband exhibited heterozygosity for a rare nondeletional alpha-thalassemia mutation at codon 59 (GGC → GAC), identified as Hb Adana. Remarkably, this particular pregnancy resulted in the birth of a phenotypically normal girl with three alpha-gene deletions.

This child represents the fifth pregnancy for a mother with a previous miscarriage and the second surviving child after birth. The mother's history included a distressing incident in 2016 involving a baby with hydrops fetalis at 33 weeks of gestation, characterized by congenital anomalies such as ascites, heart abnormalities, pleural effusion, and intrauterine fetal death. Subsequent years witnessed multiple miscarriages at 6 weeks of gestation in 2017 and 2018. In 2019, the mother carried twins, delivered via C-section, one being a male child diagnosed with alpha-thalassemia and the other a child with hydrops, who passed away immediately after birth. Intriguingly, the mother also experienced multiple episodes of pre-eclampsia in 2021, which were effectively managed.

The patient was born in 2021 at 35 weeks of gestation, weighed 2.2 kg at birth, and her APGAR Score at birth was 6. She received intrauterine transfusions at 21 and 25 weeks of gestation. After birth, she spent 3 weeks in the neonatal ICU, where a portal catheter was successfully inserted without complications, and she was intubated because of low oxygen saturation.

During her hospital stay, she underwent two additional transfusions. After discharge, she consistently received blood transfusions every 4 weeks for the past 6 months and was currently receiving iron supplementation. This intricate case underscores the complexity and challenges associated with α-thalassemia, necessitating comprehensive medical care and ongoing interventions to effectively manage this condition.

Investigations

Genetic testing

Mother: Alpha thalassemia minor trait with Southeast Asian deletion.

Father: Hb Adana deletion, normal Hb, low MCV, and mean cell Hb.

Patient: Southeast Asian deletion, combined with Hb Adana.

Blood typing

Mother: O positive.
Father: B positive.
Patient: O positive.

Post-transfusion Hb fractionation

EDTA whole blood Hb A: 95.6%.
Hb F: 1.3%.
Hb A2: 2.6%.
Hb variant Z12: 0.5%.

Hb pattern and concentration

The overall pattern shows Hb variants in Z12.
Elevated Hb F.

Final diagnosis

Transfusion-dependent anemia alpha thalassemia major.
Intrauterine Hb Barts consistent with Hydrops Fetalis.

Discussion

In this case report, we present a comprehensive analysis of two significant hemoglobinopathies: Hb Adana and Hb SEA. Our exploration delves into the molecular intricacies and clinical manifestations of these conditions, emphasizing the diagnostic challenges and the critical role of accurate genetic counseling.

Nondeletional alpha thalassemia mutation Hb Adana is a kind of the gene GGC → GAC, which results in Gly → Asp replacement at codon 59 of the HBA1 or HBA2-globin gene[21]. This replacement entails an excess of glycine at an E helix site that is tightly coupled to a B helix glycine residue. The stability and integrity of the cell molecule are drastically altered by this substitution, which results in aberrant precipitates on the red cell membrane, which induces hemolysis and inefficient erythropoiesis[22]. According to previous reports, this mutant variation is associated with a common α1-thalassemia deletion, which causes a severe form of hemolytic H illness with anemia[23]. The clinical manifestation of Hb Adana in patients varies depending on the quantity and type of the affected alpha-globin genes. Notably, a distinction is observed between individuals with homozygous alpha 1 (ααAdana/ααAdana) and homozygous alpha 2 Adana (αAdanaα/αAdanaα). Homozygous alpha 1 results in mild anemia, while homozygous alpha 2 leads to hydrops fetalis in multiple cases. Compound heterozygosity, particularly with the co-inheritance of an α0 gene deletion, gives rise to a spectrum of phenotypes, including hydrops fetalis. Other instances involving alpha globin deletions manifest varying degrees of severity, ranging from mild anemia to severe HbH-like disease[24–27]. Contrary to the conventional alpha thalassemia paradigm, Hb Adana associated with hydrops fetalis challenges the notion that four alpha gene deletions are required for this condition. Instead, Hb Adana demonstrates a nonstandard genotype-phenotype correlation, akin to other Hb variants such as Hb CS or Hb QuongSze[24–30].

The less severe variants of α-thalassemia, represented by heterozygous states for deletional (–α/αα) and nondeletional (αNDα/αα) α-thalassemia, exhibit very minor decreases in red cell indices that overlap with normal values. Hb Bart's is slightly higher in some, but not all, newborn babies. It diminishes throughout the first year of life and is not replaced by Hb H. The homozygous status for these disorders is defined by moderate hypochromic microcytic anemia at birth, marked by considerably lower MCV and mean corpuscular hemoglobin (MCH) levels as well as higher Hb Bart levels[11]. Hb H–Constant spring patients frequently experience severe developmental delays, iron overload, and repeated transfusions throughout the first 10 years of life[12,19,20,31–33]. They are particularly vulnerable to abrupt and severe anemia after a feverish illness. These infections are frequently minor. When they were 10 years old, most patients had repeated transfusions. Usually, hemolytic and acute anemic episodes are accompanied by increases in reticulocyte and bilirubin levels. Twenty percent of acute anemia episodes are hypoplastic and caused by viral infections, such as parvovirus. In addition to fever episodes, sudden drops in Hb levels are frequently linked to pregnancy[11]. Splenomegaly may be correlated with the clinical severity of nondeletional Hb H illness, as it is more frequent in this condition[11].

Our research revealed that cases of Hb Adana associated with hydrops fetalis deviate from the classical alpha-thalassemia paradigm. This deviation necessitates a nonclassical genotype-phenotype correlation, akin to other hemoglobinopathies such as Hb CS or Hb Quong Sze. In such cases, specific combinations of nondeletional and deletional mutations can lead to severe presentations[27–30].

Although carriers often display normal or subtle hematological abnormalities, the diagnosis of Hb Adana poses a considerable challenge. The frequency of Hb Adana varies across countries and ethnicities within the thalassemia belt, with underestimations likely attributable to limited access to accurate genetic diagnoses in specific regions[24–27].

One of the most prevalent forms of α-thalassemia among Chinese and Southeast Asian populations is SEA deletion α0-thalassemia. The cause of it is a 20.5 kb DNA loss on chromosome 16 that eliminates the Ψα2-, Ψα1-, α2-, α1-, and θ1 globin genes[34,35]. When both parents carry this α0-thalassemia, there is a 25% chance that the fetus will have homozygous α0-thalassemia, resulting in Hb Bart's hydrops fetalis syndrome, which is associated with poor prognosis. In most cases, intrauterine mortality or death soon after birth occurs due to anemia, cardiovascular issues, and other severe features of this syndrome, including hemochromatosis[8,29,36].

While there have been reported cases of survivors with Hb Bart's hydrops fetalis syndrome, this condition can cause major pregnancy-related difficulties for mothers, including hypertension, pre-eclampsia, polyhydramnios, and severe postpartum hemorrhage[37,38].

Three distinct families with homozygosity of Hb Adana (αcd 59 α/α cd 59 α) presented with hydrops fetalis fetuses, despite possessing two functional α-globin genes, according to a case report from Indonesia. Pregnant women with the –-SEA/– –SEA or (αcd 59 α /– –) genotype who miscarry early in gestation were shown to have less severe clinical signs of hydrops fetalis. According to this study, the compound heterozygote for Hb Adana on the α1-globin gene and α0-thal did not exhibit hydrops fetalis; instead, they had severe hemolytic anemia that required frequent blood transfusions starting at a young age[26,39].

A 29-year-old lady in her third pregnancy was the subject of a case report. Her two prior pregnancies were affected by early neonatal death at 21 and 28 weeks of gestation due to hydrops fetalis. The patient was shown to have a heterozygous (--SEA) α-

gene deletion through DNA analysis, but her spouse had compound heterozygosity for both the α3.7 deletion and the codon 59 (GGC→ GAC) mutation of the α-gene[40].

One-year-old girl with an atypical form of alpha-thalassemia was reported in another case report. The husband was found to be heterozygous for Hb Adana, while the mother was heterozygous for a south-east Asian (SEA) α-gene deletion[41].

Antenatal diagnosis is crucial and involves gap PCR exploration of fetal tissues and analysis of fetal blood samples via cordocentesis[42]. This shows the pivotal role of hematologists in evaluating patients with unexplained anemia and/or microcytosis. Therefore, comprehensive evaluation of α-thalassemia, including α-globin gene sequencing, is recommended. Hematologists play a vital role in ensuring accurate diagnosis and genetic counseling in high-risk patients and in preventing complications, such as hydrops fetalis.

Accurate understanding and differentiation of α-thalassemia depend heavily on knowledge of the molecular mechanisms that control the expression of -globin genes. Premarital and postmarital screening programs and baby screening are available nationwide. Identification of at-risk families and population screening are necessary for thorough thalassemia investigations. Modern DNA analysis, Hb fractionation, peripheral blood film morphology, total blood count examinations of Hb levels, and red cell indices will aid simple discrimination[24,30,35].

Because of the possibility of comparable hematological characteristics in carriers of α + -thalassemia, especially those carrying the -α3.7 allele, DNA analysis is crucial for the correct diagnosis. The interaction between deletional and nondeletional types of α-thalassemia, α-globin gene mismatches, and β-globin gene deletions (leading to β-thalassemia, HbS, HbE, etc.) might have a considerable influence on the hematological and clinical severity observed in the majority of individuals. To accurately investigate thalassemia, hematological examination, Hb fractionation using a reliable method, molecular analysis of alpha-globin genes, and gene clustering by direct sequencing are advised[24–30].

A 64-year-old Greek woman was found to have a late mutation in a previous case. It was emphasized how important it is to perform DNA investigations on patients whose phenotype and routine test findings are inconsistent or who arrive with severe, late problems, in addition to molecular testing to confirm common mutations, such as the –a3.7 kb deletion[43].

In general, genetic counseling for hereditary Hb opathies is critical to reduce the development of Hb Bart's hydrops fetalis syndrome, which can result in newborn mortality and major health problems in the mother during pregnancy. Genetic counseling will only make sense if the clinical outcome correctly distinguishes between the α-globin genotype and other genotypes associated with the same symptoms as those observed in various types of α-thalassemia. The identification of numerous genetic modifiers of α-thalassemia has become easier with recent advancements in genetic counseling, and their effects on various phenotypes have been recorded. Therefore, relatively inexpensive high-throughput DNA-testing methods are widely available[25–27,42,44].

More public lectures and seminars are needed to help individuals with thalassemia and their families learn more about the illness and address the social and religious barriers that can be avoided to prevent it. Health education regarding thalassemia prevention and awareness is also needed. The disorder's importance in people's daily lives will become clear to them. Therefore, education of the public with community support is beneficial. A comprehensive preventive strategy that incorporates genetic testing, prenatal profiling, premarital counseling, and community-wide awareness campaigns, with a special emphasis on societal and religious barriers, may lower the incidence rate. This can help avoid thalassemia in nations with low resources. Nationwide premarital screening initiatives should be targeted to increase public knowledge of thalassemia[45]. The earliest possible diagnosis of congenital abnormalities during pregnancy is achievable with early prenatal screening and detection. Two varieties of tests were conducted: screening tests and diagnostic tests. It is recommended that all pregnant mothers undergo prenatal screening. Testing options for determining the condition's risk include both invasive and noninvasive procedures. A diagnostic test should be performed to confirm the existence of a genetic disease in either partner when the screening test is positive. Cells obtained from the fetus are used for diagnostic testing using chorionic villus sampling or amniocentesis[46]. In 90% of cases, Doppler ultrasonography of the middle cerebral arteries can identify anemia and hydrops fetalis later in pregnancy. Once a pregnancy reaches 16 weeks of age, cerebral artery measurements can be performed consistently to identify intrauterine anemia. Increasing evidence points to fetal survival without intrauterine transfusions. These infants are more susceptible to significant neurocognitive impairment and have serious neonatal problems. These problems seem to have been alleviated by intrauterine transfusions. However, these babies require stem cell transplantation or lifetime transfusion[11].

Conclusion

More research on this condition is emerging, suggesting that nondeletion HbH sickness is becoming more common and exhibits a wider range of clinical manifestations, in addition to the possibility that it is a more harmful and less benign variant of the α-thalassemia syndrome than previously thought. The molecular and phenotypic expressions of this disease appear to differ. There is a broad range of gene mutations and many clinical presentations associated with HbH disease, but only a handful of these are linked to negative results.

Our case report and review effectively synthesize information on Hb Adana and Hb SEA, shedding light on diagnostic complexities, the imperative need for accurate genetic assessment, and the pivotal role of hematologists in managing these conditions. We offer valuable insights into genotype-phenotype correlations and stress the significance of tailored genetic counseling for high-risk populations. Further large-scale studies on these various geno-pheno presentations are essential to guide hematologists decisions, methods for diagnosis of such conditions, improvement of illness outcomes, and promotion of preventive measures.

Ethical approval

The patient guardians signed full informed consent and were reviewed and approved by both the NMC Central Scientific Committee (NMCHC/AUH/CSC/APP/002) CSC Proposal number (NMCHC /CSC/2023/0028), and NMC Ethics Committee Number (NMC/PREC/AUH/2-23/ 0029). All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and the 1964

Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent

Written informed consent was obtained from the patient's parents/legal guardian for publication and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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Author contribution

Y.A., M.M.B., S.A., S.J., and W.H.: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft, writing – review and editing; visualization, supervision, and project administration.

Conflicts of interest disclosure

The authors declare no conflicts of interest.

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This is a case report, informed consent, and IRB CSC, and Ethic approvals are available, mentioned.

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