



ORIGINAL ARTICLE

Clinical consequences of alpha-thalassemia in the Basque Country, Spain. Impact of neonatal screening^{☆,☆☆}



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Abstract

Introduction: Alpha-thalassemia is the most common hemoglobinopathy with a variable clinical manifestation depending on the number of allele mutations (asymptomatic/mild anemia if 1–2 allele mutations, severe disease if 3–4 allele mutations). A study was conducted from May 2011 on hemoglobinopathies found in the neonatal screening in the autonomous community of the Basque Country (CAPV).

Objectives: To analyze the impact of alpha-thalassemia in this area and the effectiveness of its neonatal screening.

Methods: A review was made of patients with a positive gene study for alpha-thalassemia over a 2-year period (2012–2013) and an analysis was made of the age at diagnosis, ethnic group, analytical result, and treatment.

Results: The genetic study was performed on 107 patients, of which 61 had some mutation, with 62% having one allele mutation and 38% with two alleles. The mean age at diagnosis was 31 years, with 28% being younger than eighteen years old. Most of the patients were European with a significant number of Africans (26%) and Arabs (13%). All patients were asymptomatic, and 28% had mild anemia. Two patients were diagnosed by neonatal screening. Most of them did not need any treatment or only required iron therapy.

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PALABRAS CLAVE

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Conclusions: The detection of one or two alpha gene mutations has no clinical impact, but allows genetic counseling. No patient was found with 3–4 mutations or severe symptoms in our region. Contrary to the diagnosis of other diseases, our results do not support that routine neonatal screening for alpha-thalassemia has any clinical impact in our community.

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Repercusión clínica de la alfa-talasemia en nuestro medio. Impacto del *screening* neonatal

Resumen

Introducción: La alfa-talasemia es la hemoglobinopatía más frecuente de expresión clínica variable en función del número de alelos mutados (1–2 alelos mutados: asintomático/anemia leve, 3–4 alelos mutados: enfermedad grave). Desde mayo de 2011 se ha añadido el estudio de hemoglobinopatías al *screening* neonatal en la Comunidad Autónoma del País Vasco (CAPV).

Objetivos: Valorar el impacto de la alfa-talasemia en nuestro medio y la utilidad del *screening* neonatal en su detección.

Método: Revisión de pacientes con estudio genético positivo para alfa-talasemia durante 2 años (2012–2013) y estudio de la edad al diagnóstico, etnia, resultados analíticos y tratamiento.

Resultados: Se realizó un estudio genético de alfa-talasemia a 107 pacientes, de los cuales 61 presentaron alguna mutación. El 62% tenía un alelo mutado y el 38%, 2 alelos. La edad media al diagnóstico fue de 31 años, con un 28% menores de 18 años. La mayoría eran de procedencia europea con un porcentaje no desdeñable de africanos (26%) y árabes (13%). Todos los pacientes estudiados estaban asintomáticos con anemia leve en el 28%. Dos pacientes fueron diagnosticados por *screening* neonatal. La mayoría de pacientes no requirió tratamiento o precisó ferroterapia.

Conclusiones: La presencia de una o 2 mutaciones en los genes alfa carece de repercusión clínica, y el único interés de su estudio es que permite el consejo genético. En nuestro entorno no hemos encontrado pacientes con 3–4 mutaciones ni con sintomatología grave. A diferencia de lo que ocurre con otras enfermedades, nuestros resultados no apoyan que el *screening* neonatal de alfa-talasemia tenga un impacto significativo en nuestro entorno.

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Introduction

Thalassaemias are inherited disorders of hemoglobin synthesis. The most common type of hemoglobin at birth is hemoglobin A, which is composed of four polypeptide chains, two alpha (α) chains and two beta (β) chains. In alpha-thalassaemia, there is reduced or absent production of the alpha chain, and consequently a relative excess of beta chains (gamma chains in the newborn). Excess beta chains can form soluble tetramers (HbH), but these are unstable and precipitate, giving rise to symptoms (peripheral haemolysis and, to a lesser degree, ineffective erythropoiesis).^{1,2}

Each copy of chromosome 16 has two pairs of genes that encode the alpha chain, so the normal genotype is $\alpha\alpha/\alpha\alpha$. The main mechanism leading to alpha-thalassaemias is the partial or total deletion of a gene.³ At least 128 different genetic defects that may cause alpha-thalassaemia have been identified to date.^{4,5} We also know of at least seven forms of alpha-thalassaemia that are due to

nondeletional mutations, which usually carry more severe symptoms. The most frequent deletion in Spain involves 3.7 kb of DNA ($-\alpha^{3.7}$), which through homologous recombination leaves a single functional alpha gene in the affected chromosome^{4,6,7}:

- Loss of the 4 alleles ($-/-$) leads to a total absence of alpha chains, which is incompatible with life (Hb Bart syndrome).
- Deletion of 3 alleles ($\alpha-/-$) is also known as hemoglobin H disease (HbH) because it leads to the formation of tetramers of excess beta chains and causes moderate to severe haemolytic anemia, ineffective erythropoiesis, splenomegaly and bone changes.
- Deletions of 1 ($\alpha\alpha/\alpha-$) or 2 ($\alpha\alpha/-$ or $\alpha-/\alpha-$) alleles are known as silent alpha-thalassaemia and alpha-thalassaemia trait, respectively. Patients are usually asymptomatic, but may have mild microcytic and hypochromic anemia. These forms are more prevalent in individuals of Asian or African descent.^{1–4}

Alpha-thalassaemia is probably the most common haemoglobinopathy, with a global distribution that affects 5% of the world's population, although it is most prevalent in specific regions. Alpha-thalassaemia seems to confer some protection against severe malaria (especially malaria caused by *Plasmodium falciparum*) and this is probably why it affects up to 90% of the population in regions like Papua New Guinea or Ghana. Alpha-thalassaemia is also associated with other haemoglobinopathies, such as sickle-cell anemia, hemoglobin E disease or beta-thalassaemia. Anemia is usually more severe in patients that have more than one haemoglobinopathy compared to those that only have one disorder. In Oman, the prevalence of alpha-thalassaemia is 48%, the prevalence of sickle-cell disease is 5.7% and that of beta-thalassaemia is 2.5%.^{8,9}

In May 2011, haemoglobinopathy testing by means of electrophoresis was added to the neonatal screening program of the Autonomous Community of the Basque Country. The purpose of our review was to assess the clinical impact of alpha-thalassaemia in our region and the impact that neonatal screening has had on this autonomous community.

Materials and methods

We reviewed the medical records of pediatric and adult patients who had a genetic test for alpha-thalassaemia requested by different parties in collaboration with the Servicio de Documentación Médica (Medical Records Department) of the Hospital Universitario de Cruces, over a period of 2 years (2012–2013).

We collected epidemiological and clinical data from the medical records and the Global Clinic database of the Osakidetza (Basque Health Service).

We reviewed the complete blood counts performed in our hospital from the date that genetic testing was requested due to suspicion of alpha-thalassaemia to the present, using the Omega 3000[®] software, and including the data for abnormal electrophoresis tests obtained in the neonatal screening.

We collected the results of DNA testing (simple 3.7 and 4.2 kb deletions in the alpha-globin locus of chromosome 16), performed by polymerase chain reaction (PCR) at the reference laboratory.

We designed a database that included the following variables: age at diagnosis, ethnicity/country of origin, reason the patient was referred for testing, laboratory results of the complete blood count and genetic testing, and treatment received.

Results

Genetic testing for alpha-thalassaemia was performed in 107 patients, and some type of mutation was detected in 61, which amounted to 57% of the analyzed samples.

Tests were performed to detect two simple deletions in the alpha-globin locus of chromosome 16: a 3.7 kb deletion and a 4.2 kb deletion. All mutations found were due to a 3.7 kb deletion, and none were due to a 4.2 kb deletion. Sixty-two percent (29) corresponded to heterozygous mutations ($-\alpha^{3.7}/\alpha\alpha$; silent alpha-thalassaemia), while 38%

(32) corresponded to homozygous mutations ($-\alpha^{3.7}/-\alpha^{3.7}$; alpha-thalassaemia trait).

The mean age at diagnosis was 31 years, and the median age was 33 years. The range was 0–79 years. Seventeen patients (28% of the sample) were younger than 18 years.

The ethnicity of patients with alpha-thalassaemia mutations was as follows: 59% (32) were of European descent, 26% (14) of African descent, 13% (7) were of Middle Eastern origin and 2% (1) were from South America.

All patients in the study were asymptomatic or had nonspecific manifestations such as asthenia. Genetic testing was performed during the investigation of microcytic anemia/microcytosis. Only two patients were diagnosed through a positive result in the neonatal screening, and both had sickle-cell anemia. Twenty-two percent (12) of the patients had a family history of alpha-thalassaemia. Of all patients, 7.5% (4) were also HbS carriers.

The laboratory results obtained at the time of diagnosis were: 28% (15) of patients had anemia (45% [9] of patients with homozygous mutations and 18% [6] of patients with heterozygous mutations). The mean hemoglobin level in patients with homozygous mutations was 11.7 g/dL, and the lowest level was 8 g/dL. In patients with heterozygous mutations, the mean level was 12 g/dL and the minimum was 8.7 g/dL. Seventy-eight percent (42) of patients had microcytosis, corresponding to 72% (23) of patients with homozygous mutations compared to 65% (19) of patients with heterozygous mutations. Of all patients with alpha-thalassaemia mutations, 25% (15) had iron deficiency, corresponding to 22% (7) of patients with homozygous mutations versus 38% (11) of patients with heterozygous mutations. Another two patients (3%) had folate deficiency.

As for treatment, 68% (41) of the patients required none, 18% (11) received iron therapy, 3% (2) were treated with B₁₂, and 1.5% (1) was treated with folic acid. Two patients required transfusions, one of them in the context of pancytopenia secondary to dengue fever and the other for an autoimmune haemolytic anemia exacerbation, with no relationship with their alpha-thalassaemia carrier status.

Discussion

Diseases associated to alpha-globin abnormalities are frequently found in tropical and subtropical regions. The selection in these regions is partly due to the protection they confer against malaria, although the mechanism of this protection is not yet understood. Alpha-thalassaemia is the most prevalent form, and some individuals have several associated disorders (such as beta-thalassaemia or sickle-cell anemia). Hemoglobin H disease is most prevalent in Southeast Asia, the Middle East and the Mediterranean region, while hydrops fetalis syndrome is most prevalent in Southeast Asia.¹

The low clinical and hematological impact of some alpha-thalassaemia genotypes makes it hard to establish their actual prevalence, and they are most frequently suspected in patients with asymptomatic microcytic anemia.

Of the patients with genetic abnormalities included in our sample, none required intensive care (except for two that required transfusions for acute illnesses unrelated to alpha-thalassaemia), and oral replacement therapy was only

initiated in 24%. Most mutations involving one or two alpha-globin genes did not have clinical impact nor required treatment, and their identification was only relevant for the purposes of genetic counseling.

Historically, symptomatic alpha-thalassaemia has been an uncommon disease both in Spain and in the United States. However, its prevalence in certain regions of the United States has been increasing in recent years, especially among Asian immigrants.² The evidence suggests that early diagnosis is important to better manage these patients, so the possibility of performing neonatal screenings has been considered in that region. Our review did not identify any patients suffering from symptomatic thalassaemia (mutations in 3 or 4 of the alpha-globin genes) and only two patients were diagnosed with alpha-thalassaemia by means of neonatal screening. Unlike what has happened with other diseases, our results did not support the hypothesis that early diagnosis by means of neonatal screening for alpha-thalassaemia would be clinically relevant in our region.

Conflicts of interest

The authors have no conflicts of interest to declare.

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