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Alpha- Thalassemia: An Overview

Hayaa M. Alhuthali[1*]

[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, P. O. Box 11099, Taif 21944, Saudi Arabia

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***Corresponding author:** Hayaa M. Alhuthali
Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, P. O. Box 11099, Taif 21944, Saudi Arabia

Abstract **Review Article**

Alpha-Thalassemia is the commonest hereditary monogenic disease worldwide. α-thalassemia is caused by α-globin gene losses and categorized into α-thalassemia 1 and α-thalassemia 2 depending on how many α-globin genes are functioning. A thalassemia 1 is defined by inactivation of both α-globin genes on a chromosome, while in α-thalassemia 2, one gene is active. The clinical phenotype depends on the degree of genes impairment. This review will present an overview of α-thalassemia, its incidence, causes, and clinical characterization, and discuss different laboratory techniques used for the diagnosis.

Keywords: Alpha-Thalassemia, hereditary monogenic disease, α-globin gene, hemoglobinopathy.

INTRODUCTION

Each year, more than 300,000 people worldwide are seriously affected by (Lee *et al*., 2019) thalassemia. Alpha-thalassemia (α-thalassemia) is one of the most common hemoglobinopathy disorders involving the genes HBA1 and HBA2 and affects 5% of the world's population (Olwi *et al*., 2018; Borgio, 2015a). α-thalassemia is an autosomal recessive disease characterized by impaired hemoglobin production. [7] Normal hemoglobin (HB) consists of two alpha (α) chains and two beta (β) protein chains and makes up 95%-98% of adult hemoglobin. The quantitative abnormalities in the alpha-globin (α-globin) chain production (Yatim *et al*., 2014) result in fewer normal HB and unstable beta-globin molecules, predisposing red blood cells to destruction.

α-thalassemia is caused by defective protein synthesis due to mutations in the α-globin genes. The α-globin genes are duplicated and localized in a cluster in the telomeric region of the 16p13.3 chromosome (Dehbozorgian *et al*., 2015). Each copy of the chromosome carries two functional α-globin genes that code for the chains that serve as components in fetal and adult hemoglobin (Silao *et al*., 2021).

Typically, there are four genes (α1α2/α1α2), which correspond to four α-globin proteins. Different kinds of α-Thalassemia originate from losing one or more of these genes. The α-globin protein is a subunit of hemoglobin, a bigger protein found in red blood cells (RBCs) and responsible for transporting oxygen through the body. The α- 1 gene and two α- genes produce similar α-globin proteins (Borgio, 2015a), but the transcription levels differ. The α- 1 gene encodes two to three times less than the α- 2 globin gene. The two α- genes' expression implicates the amount of hemoglobin variant and the pathophysiology of the non-deletional and deletional forms of α-thalassemia.

This review focuses on α-thalassemia and will present the current literature on α-thalassemia, including the incidence, classification, and clinical characterization, and discuss different laboratory techniques used for the diagnosis.

Incidence of Alpha Thalassemia

Thalassemia affects approximately 20% of the world's population. Every year, over 56,000 babies worldwide are born with severe α-thalassemia, with more than half requiring frequent transfusions. In addition, approximately 5,500 prenatal annual deaths are caused by hydrops fetalis (Lee *et al*., 2019). It is widespread in the Indian subcontinent, Mediterranean countries, the Middle East, Southern China, and Southeast Asia (Huang *et al*., 2021). In the Mediterranean and Middle East, up to 40% of people are α-thalassemia carriers (Vijian *et al*., 2021). The frequency of thalassemia in Saudi Arabia is among the highest in the world, ranging from 0.4 percent to 5.9

percent (Olwi *et al*., 2018). Thalassemia is prevalent in Gulf Cooperation Council (GCC), and its epidemiology has been reviewed recently. Abu-Shaheen *et al*., (2020) reported that the incidence of thalassemia among children younger than five years and adult patients ranged between 0.25% - 33% and 0.035% to 43.3%, respectively. In contrast, the prevalence among children aged more than five years was 0.9%.

1.2.3 Alpha Thalassemia's Causes

α-thalassemia is classified as α+ or α0-thalassemia. α+-thalassemia (or α-thalassemia 2) is defined by the absence or reduction of the expression of a single α-globin gene because of either a point mutation (non-deletional α+-thalassemia) or deletion (deletional α+-thalassemia). The mutated allele can be homozygous or heterozygous for α+-thalassemia. While in α0-thalassemia (or α-thalassemia 1), the duplicatedα-globin genes are equally deleted on one allele, and thus there is no difference between deletional or non-deletional form (Munkongdee *et al*., 2010; Farashi & Harteveld, 2018a).

95% of α-thalassemia are due to deletional mutations, while the non-deletional alleles are causative for the rest (Pornprasert *et al.*, 2018). The most common deletional form of α+-thalassemia is -α3.7. It has been reported that the primary reason for α-thalassemia worldwide is a loss of 3.7 kb of DNA induced by base pairing between mismatched chromosomes. This aberration affects both genes in cis and results in a unique hybrid gene (α2α1) (-α3.7 deletion) (Borges *et al.*, 2001). The non-deletional alleles of α-thalassemia commonly affect the termination codon (TAA), promoter (GT/ AG), initiation codon (ATG), polyadenylation signal (AATAAA), and splicing signals. Polyadenylation site mutations α2 AATA—α2 AATGAA and α2 AATAAG (in the Mediterranean and Middle East deletions) and αIVSI(−5nt) α (in the Mediterranean deletion) are the most frequent non-deletional mutations (Harteveld *et al*., 1994).

A previous study in Western Australia, which has had many refugees from Southeast Asia, detected single and double α-globin gene deletions and mutations (Prior *et al*., 2004). Of whom, 35.4% were with an anomaly in the α-globin gene, 50.6% were with a single gene deletion (α2-thalassemia), mostly -3.7 kb, and 31.2% were with double α-gene deletions (α1-thalassemia), including 7 cases of hemoglobin H (HbH) disease (Prior *et al*., 2004).

In Saudi Arabia, the most often observed deletions and point mutations in α-thalassemia patients are - α 3.7 heterozygous and α^{PA-1} mutation (Borgio, 2015a).

In Kuwait, the α^{PA-1} was the prevalent mutated allele and the most common cause of HbH disease, followed by the –α-3.7 deletional allele, which was usually existing as a compound heterozygote with other alleles (Adekile *et al*., 2020). The α0 (--MED) allele was reported to be also frequent among Kuwaiti patients. It appeared as heterozygotes in combination with other mutated alleles such as $\alpha2^{cd19-G}$, $\alpha^{IVS1-5nt}$, -α3.7, and α^{PA-1}. Patients with the --MED/ $\alpha^{cd19}\alpha$ genotype were with severe anemia and on regular transfusions (Adekile *et al*., 2020).

The α^{PA-1} allele is widespread in the GCC and primarily associated with HbH disease in the Kingdom of Saudi Arabia (Al-Awamy, 2000a, Abdulla Alharby *et al*., 2022), Jordan (Ghoush, 2008), Kuwait (Adekile *et al*., 2020), UAE (El-Kalla & Baysal, 1998) and Bahrain(Jassim *et al.*, 2001; Al Moamen *et al*., 2018). Borgio (2015b) reported the identification of novel mutations, including Cd39(C→T), IVS I-5(G→C), c.848T>C, and c.623delA on ATRX gene in the Saudi population that contributes to α-thalassemia like phenotype.

In addition, the α-thalassemia determinants were studied and characterized on the main island of Penang, where Malays represent most of the population (Yatim *et al*., 2014). Out of the 13 distinct α-thalassemia determinants investigated, two types of deletions and two types of mutations were found (Yatim *et al*., 2014). The most common aberrations were the Southeast Asian deletion α-thalassemia 1 (--SEA), which was found on 20 α-globin haplotypes, and chain termination mutant ($\alpha^{CS}\alpha$), with a frequency of 35.7% and 30.3%, respectively. The remaining two alleles, -α3.7 and α^{Cd59}, were less common and found in lower numbers (Yatim *et al*., 2014). Furthermore, a reverse dot blot for all point mutations and a gap-polymerase chain reaction was used to examine common α-globin mutations in a 4010 Iranian population (Dehbozorgian *et al*., 2015). The most frequently mutated allele was the -α3.7 deletion (43.84%), followed by the $\alpha^{IVS1/5nt}$ mutation (4.91%) and Hb Constant Spring (Hb CS, codon 142, TAA>CAA(alpha2)) (2.78%) (Dehbozorgian *et al*., 2015).

1.2.4 Clinical characterization of α-thalassemia

The alpha globin gene has four alleles, and the severity of the disorder varies depending on how many alleles are defective (Bajwa & Basit, 2021). The normal balance of four functioning α-globin genes can be altered by the loss/ inactivation of one, two, three, or all four copies, which explains clinical variance and illness severity (Qiu *et al.*, 2013).

The deletion of one α-globin gene causes a silent carrier, while twice deletion, either in cis or trans (−−/αα, −α/−α) of α-globin genes, respectively, causes thalassemia traits (Kwaifa *et al*., 2020). No apparent clinical symptoms are associated with inheriting one or two aberrant α-globin genes (Kwaifa *et al*., 2020). However, the inheritance of three aberrant α-globin genes causes HbH disorder associated with moderate to severe symptoms and the need for blood transfusions or

iron chelation therapy on rare occasions (Lithanatudom *et al*., 2016). The most severe type of α-thalassemia is Hemoglobin Bart's hydrops fetalis disorder (also called α-Thalassemia major). Hydrops fetalis is defined by the loss of all four α-globin genes, and almost all affected newborns die in utero (Lithanatudom *et al*., 2016).

In the non-deletion type, a patient has two α-globin genes, but one has a non-deletion defect, such as a point mutation. The degree of clinical manifestation in non-deletion is also determined by whether the mutation partially or entirely prevents the formation of the remaining normal alpha chains.

There is a good correlation between the degree of imbalanced α- globin chains and disease severity. Non-deletional variants of α+-thalassemia can result in a greater decrease in the synthesis of α-globin chain compared to the deletional forms, correlating with the clinical phenotype (Farashi & Harteveld, 2018b). Non-deletional HbH disease patients have more serious clinical symptoms, are more anemic, more likely to develop hepatosplenomegaly, and require frequent blood transfusions (Kanavakis *et al*., 2000). A compound heterozygosity for both non-deletional type of α-thalassemia 2 and deletion forms of α-thalassemia 1 cause serious symptoms than deletional types of α2- and α1-thalassemia, especially in the case of HbH disorder (Farashi & Harteveld, 2018b)**.**

1.2.5 Laboratory Diagnosis of alpha thalassemia

The majority of α-thalassemia characteristics are asymptomatic and are discovered during routine hematological tests. However, a decrease in Hb level can occur when there is concurrent blood loss, infection, poor nutrition, or any illness that might cause clinical anemia (Vijian *et al.*, 2021). Therefore, a combination of different assays is required for α-thalassemia diagnosis. These laboratory tests include complete blood count (CBC), blood smear and Hb quantification, and DNA testing (Brancaleoni *et al.*, 2016).

1.2.5.1 RBC indices of alpha thalassemia

Hematological indicators, which include a precise blood count using an electronic cell analyzer, are the initial screening test. Thalassemic patients are characterized by microcytic and hypochromic red blood cells (RBC), changes in the level of hemoglobin (Hb), mean corpuscular volume (MCV), and mean corpuscular Hb (MCH).

Microcytic anemia is caused by reduced Hb production due to defective globin chain production in thalassemia patients (Vijian *et al*., 2021). RBC indices, particularly MCV, can be affected by several disorders, including iron deficiency anemia (IDA) and inflammation, and these levels may be as low as those discovered for thalassemia traits. As a result, in a population where α-thalassemia is highly prevalent, screening for thalassemia carriers using only RBC indices is insufficient (Viprakasit & Ekwattanakit, 2018). Low Hb, MCV and MCH cannot distinguish the thalassemia from other disorders or differentiate the α- from the β-thalassemia (Vijian *et al*., 2021). Thus, individuals with MCV less than 80 FL and MCH less than 27 pg should be tested further to confirm the diagnosis of α-thalassemia (Vijian *et al.*, 2021) (Sanchaisuriya *et al*., 2005).

Red cell morphology alterations can be seen in most thalassemia carriers. The most common modifications are microcytosis, hypochromia, and anisopoikilocytosis. Basophilic stippling and the presence of specific target cells are two less common observations (Brancaleoni *et al*., 2016).

1.2.5.2 Hb quantification

A hb analysis is performed using high-performance liquid chromatography (HPLC) or capillary zone electrophoresis (CE) systems. They have an excellent resolution, quantification, and reproducibility of several abnormal and normal hemoglobin leading to an accurate diagnosis of α-thalassemia. These two technologies allow for the sensitive and precise qualitative and quantitative investigation of Hb contents. Also, they are effective methods for prenatal and postnatal thalassemia diagnosis quickly (Vijian et al., 2021). Both systems detect Hb Bart's, HbH, and HbCS in HbH and HbH-CS disorders. Additionally, they identify the most clinically significant variants and strongly correlate with diagnosing adult thalassemia (Munkongdee *et al*., 2020).

Healthy adult had normal level of Hb concentration (12 g/dl), MCH (27 pg), MCV (80 fL), and had 3.5%of HbA2. Carriers of thalassemia have a normal Hb concentration but present low MCH and MCV. Hb A2 quantity discriminates the alpha carriers, who have normal Hb A2 (3.5%), from the β-thalassemia carrier, which presents a high level of Hb A2 (> 3.5%). Table 1 summarizes the percentage of Hb variants in different phenotypes using HPLC and CE systems (Munkongdee *et al*., 2020).

Table 1: Hemoglobin variants in adult blood (Munkongdee *et al*., 2020)

Phenotype	Number	Hb Type	Hb A_2 %		Hb E %		Hb F %	
			HPLC	CE	HPLC	CE	HPLC	CE
Normal	45	A_2A	2.6 ± 0.4	2.5 ± 0.4	–	–	0.5 ± 0.7	0.1 ± 0.2
α-thalassemia 1 heterozygote	36	A_2A	2.3 ± 0.2	2.3 ± 0.2	–	–	0.5 ± 0.7	0.3 ± 0.5
β-thalassemia heterozygote	69	A_2A	5.5 ± 1.3	5.4 ± 0.5	–	–	1.5 ± 1.4	0.9 ± 1.4
Hb E heterozygote	85	EA	Not detected	3.5 ± 0.4	27.8 ± 7.5	25.6 ± 1.4	1.2 ± 0.9	0.4 ± 0.8
Hb E heterozygote with α-thalassemia 1 heterozygote	6	EA	Not detected	4.0 ± 0.3	21.9 ± 0.6	16.3 ± 0.8	0.9 ± 0.6	0.5 ± 0.8
Hb E homozygote	56	EE	Not detected	4.1 ± 0.8	90.2 ± 4.9	92.9 ± 3.3	4.3 ± 2.7	2.5 ± 3.1
β-thalassemia/Hb E disease	48	EF	Not detected	4.9 ± 1.6	59.4 ± 12.9	50.3 ± 13.8	31.1 ± 14.5	36.8 ± 13.3
Hb H disease	26	A_2A Bart's H	1.6 ± 1.2	1.0 ± 0.2	–	–	0.6 ± 0.6	0.2 ± 0.3
Hb H-CS disease	9	CSA_2A Bart's H	ND	0.7 ± 0.5	–	–	ND	1.0 ± 0.6
Hb CS homozygote	10	CSA_2A	ND	1.3 ± 0.6	–	–	ND	0.8 ± 0.8

Phenotype	**Number**	**Hb Type**	**Hb Bart's %**		**Hb H %**		**Hb CS %**	
			HPLC	**CE/Undetected number**	**HP**	**CE/undetected number**	**HPL**	**CE/undetected number**
Normal	45	A_2A						
α-thalassemia 1 heterozygote	36	A_2A						
β-thalassemia heterozygote	69	A_2A						
Hb E heterozygote	85	EA						
Hb E heterozygote with α-thalassemia 1 heterozygote	6	EA						
Hb E homozygote	56	EE						
β-thalassemia/Hb E disease	48	EF						
Hb H disease	26	A_2A Bart's H	Found	1.1 ± 0.7/14	Found	6.7 ± 4.8/0		
Hb H-CS disease	9	CSA_2A Bart's H	Found	4.2 ± 4.1/3	Found	11.3 ± 6.5/3	Found	2.6 ± 1.4/1
Hb CS homozygote	10	CSA_2A	Found/Not found		Found/Not found		Found	3.5 ± 2.5/0

1.2.5.3 Molecular Analysis of alpha thalassemia

DNA testing provides precise detection of thalassemia mutation. Recently, numerous DNA sequencing and polymerase chain reaction (PCR) based techniques have been available for diagnosing known and unknown mutations (Al-Jaouni, 2010). Dot blot analysis allows direct detection of minor deletions or point mutations. Non-deletion mutations of the α-globin gene are widely detected using reverse dot blot analysis and a single tube multiplex polymerase chain reaction. In RDB analysis, a single test identifies different mutations of a sample. RDB is reliable, rapid, simple and can recognize an individual's heterozygous and homozygous states. Non-deletion mutations in α-thalassemia are also detected using Sanger sequencing (Sabath, 2017).

The common deletion mutation of α-thalassemia, -α.$^{4.2}$, —SEA, —THAI, —MED, —FIL, -α.$^{20.5}$, and -α.$^{3.7}$ are currently detected by Gap-polymerase chain reaction (Gap-PCR) (Old *et al*., 2012). Multiplex Ligation Dependent Probe Amplification (MLPA) provides a rapid and quantitative analysis for less commonly occurring deletions (Al-Awamy, 2000b). There is attempts to use the amplification and direct sequencing of α-gene fragments on a large scale to improve its effectiveness and costs (Farashi & Harteveld, 2018b). Next-generation sequencing (NGS) has recently been used to look for α-thalassemia genes. Other traditional procedures may misdiagnose thalassemia, but this approach provides a reliable diagnosis. The high cost of NGS for thalassemia screening is still a significant drawback (Al-Jaouni, 2010).

CONCLUSION

α-Thalassemia is a set of hereditary blood diseases characterized by a lack of or inadequate synthesis of one or more α-globin chains. It is very heterogeneous at a molecular and clinical level. Patients with α-Thalassemia have a wide range of clinical manifestations between severe transfusion-dependent and mild or asymptomatic carrier states. A Laboratory screening test, including complete blood count, blood smear, and quantification of hemoglobin by either capillary electrophoresis or high-performance liquid chromatography, is insufficient to detect thalassemia diseases or discriminate the alpha from beta type. Molecular methods are essential for confirmatory diagnosis and effective for precise identification. The routinely employed method in the laboratory may rely on expertise, lab resources, and economic conditions.

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