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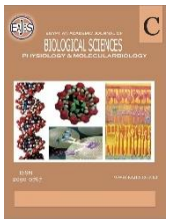

Molecular and Hematological Analysis of Alpha Thalassemia in Middle East Patients; A Cross-Sectional Retrospective Study

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ABSTRACT

Hemoglobinopathy is the most frequent genetic illness worldwide. Alpha thalassemia is common in Middle East. The loss of one or both HBA genes in the -globin gene cluster causes alpha-thalassemia. The countrywide prevalence and distribution of alpha globin gene mutations must be studied. Molecular screening and detection improve thalassemia-risk prenatal diagnosis and genetic counselling. This article compares different alpha thalassaemia mutations molecular and haematological characteristics in the Middle East. This cross-sectional retrospective analysis was carried out from September 2022 to June 2023. A study investigated 200 samples of alpha thalassemia patients in the Middle East using Reversed Dot Blot Hybridization-based multiplex-PCR to screen for 21 known α-globin gene abnormalities. We found 17 alpha-globin gene variants. The first prevalent anomaly was (deletional) 3.7 homozygous (34.5%), and the second was 3.7 heterozygous (18.5%). The genotype (--^{MED} αα /α2 poly A2) was strongly linked with lower hemoglobin and RBCs levels in α-thalassemia (p-value of 0.027 and 0.042 respectively).The most prevalent alpha thalassemia abnormality is -$\alpha^{3.7}$/ -$\alpha^{3.7}$. Alleles show diversity in Middle Eastern populations. Even genotype-matched people had different haematological parameters. Haematological criteria cannot uniquely characterize any alpha thalassaemia mutation.

INTRODUCTION

The most prevalent genetic disorder in the world is hemoglobinopathy. The morbidity and mortality rates among affected individuals are significantly impacted by thalassemia. (Weatherall, 2011).

Countries in the Mediterranean, the Indian subcontinent, the Middle East, Southern China, and Southeast Asia have a high prevalence of alpha thalassemia (α-thalassemia) (Huang *et al.,* 2021). There are 49.6 cases of sickle cell anaemia and 13.6 cases of beta-thalassemia for every 100,000 Saudis (Alsaeed *et al.,* 2018). Both sickle cell anaemia and α--thalassemia are inherited at high rates, with prevalence ranging from 0.4 to 5.9 percent in the Northern and Eastern regions, respectively (Olwi *et al.,* 2018). In recent years, premarital screening for haemoglobin abnormalities in Makkah City has revealed a 6.3% prevalence of the -thalassemia trait (Moustafa *et al.,* 2022).

The loss of either one (-) or both (--) HBA genes in the -globin gene cluster is the most common genetic cause of alpha-thalassemia. although small deletions or point mutations also contribute to the α-thalassemia mutation spectrum (Somervaille, 2001).

The α-globin genes are located on human chromosome 16 in the 16p13.3 region. There are normally 4 copies of α - globin gene (with two in each allele) (α1α2/α1α2) in an individual. The α-thalassemia phenotypes are caused by defects in one or more of the four α-globin genes (αα/αα) and autosomal recessive inheritance is the mode of transmission for these mutations (Qiu *et al.,* 2013). Based on the number of functional α-globin genes that are still present, α-thalassemia can be categorised as either α+-thalassemia or α0-thalassemia (Munkongdee *et al.,* 2010). The deletion of one pair of α-globin genes (-α/ αα) defines α+-thalassemia, whereas α0-thalassemia is characterised by double deletion (-/αα). The silent carrier condition, caused by α+-thalassemia heterozygosity, is characterised by the absence of clinical symptoms and the fact that it overlaps with normal red blood cell indices (Vichinsky, 2013). Individuals with mild microcytic and hypochromic anaemia and a normal HBA2 level are clinically characterised by the homozygosity state for α+-thalassemia and the heterozygous state for α0-thalassemia (Vichinsky, 2013). Excess β-globin chains are the hallmark of adult haemoglobin H illness (HbH disease), which is caused by the co-inheritance of these milder thalassemia types (--/-a).

Mean corpuscular haemoglobin (MCH), red cell distribution width (RDW), and mean corpuscular volume (MCV) have all been shown to have a strong correlation with the number of deleted -genes (*Full Article: The Prevalence of Alpha-Thalassemia amongst Tai and Mon-Khmer Ethnic Groups Residing in Northern Thailand: A Population-Based Study*, n.d.). Individuals with microcytosis, MCH<23.40 pg, normal values of HbF and HBA2, and no iron deficiency should not be considered to have an α0 allele, but a heterogeneous α0 allele is likely when MCV<70.80 fL and MCH <21.90 pg are present. A major predictor of haemoglobin H illness is both an MCH<19 pg and an RDW≥20% (Velasco-Rodríguez *et al.,* 2017).

The world's population, however, appears to share a few rare mutations and a few frequent ones that are region-specific (Puehringer *et al.,* 2007). There is a disproportionate number of people living with haemoglobin disorders throughout the Middle East. The people who live in the Middle East come from a wide variety of backgrounds. Therefore, it is important to assess the frequency and distribution of mutations in the alpha globin gene across the entire nation (Olwi *et al.,* 2018).

In high-risk areas for thalassemic disorders, prenatal diagnosis and practical genetic counselling may be possible through screening for and the subsequent detection of alpha gene mutations (Keser *et al.,* 2021). In thalassemia-risk areas, prenatal diagnosis and practical genetic counselling are aided by molecular screening and detection of the disease (Alhuthali *et al.,* 2023). Haematological parameters measurement is important in developing suitable molecular screening protocols to find out what genotype carriers of alpha thalassemia have (Rizo-de la Torre *et al.,* 2021).

Over 90% of alpha-globin mutations in endemic regions can be detected using the

Alpha-Globin Strip Assay, which is a rapid, simple, and reliable screening approach (Puehringer *et al.,* 2007).

Unfortunately, relatively few articles addressing the topic of molecular and haematological examination of alpha thalassemias have been published to the authors' knowledge.This article aims to ascertain the molecular varieties, and haematological profile of deletional, non-deletional and compound mutations. In addition to genotype–phenotype correlation in α-thalassemia patients with both uncommon and common mutations in a cohort of Middle Eastern patients.

MATERIALS AND METHODS

Sample Size Calculation:

Sample size was calculated by OpenEpi software (Sullivan et al., 2009), aiming for estimation of the prevalence of alpha thalassemia (available at http://www. openepi. com/ SampleSize/SSPropor.htm).

Goh, *et al.,* 2020, reported the prevalence of alpha thalassemia which was described as 22.6% (Goh *et al.,* 2020).By applying the previous figures to the equation, the minimum sample size was 190, using a power of 0.90.. The sample size was increased to 200 to increase the power of the study.

Population and Sample:

A cross- sectional retrospective study is what this present study is. The haematological and molecular data of two hundred thalassemia patients between 1 and 60 years old who attended Alborg Laboratories in Jeddah were collected retrospectively from September 2022 to June 2023. When it comes to private medical testing facilities, Al Borg Diagnostic Lab is unrivaled. It's a unified network with locations across Eastern, Western, Southern, Northern, and Central Saudi Arabia.

The research protocol was agreed upon by the Human Research Ethics Committee of Alborg Laboratories (approval reference number 11-10-2022) following regulations issued by the National Committee of Biomedical Ethics at King Abdul- Aziz City for Science and Technology. In addition, Every procedure followed the rules and regulations set out by the King Abdul-Aziz City for Science and Technology's National Committee of Biomedical Ethics.

EDTA peripheral blood was collected from those patients for Complete blood picture (CBC) and HB electrophoresis. Regarding private medical testing facilities, Al Borg Diagnostic Lab is unrivaled. It's a unified network with locations across Eastern, Western, Southern, Northern, and Central Saudi Arabia. The research protocol was agreed upon by the Human Research Ethics Committee of Alborg laboratories (approval reference number 11-10-2022) following regulations issued by the National Committee of Biomedical Ethics at King Abdul- Aziz City for Science and Technology. EDTA peripheral blood was collected from those patients for a complete blood picture (CBC), HB electrophoresis, and molecular testing of the alpha thalassemia mutation by multiplex PCR. The samples were sent to Alborg Laboratory to confirm the diagnosis of α-thalassemia through molecular testing. Microcytosis (MCV less than 80 fL), hypothermia (MCH less than 26 pg), or both without iron deficiency anaemia (IDA) were the inclusion criteria for the samples. Yonder and Pandey (Keser et al., 2021). Individuals exhibiting low red blood cell indices and aberrant iron profiles were deemed ineligible for inclusion in the study, included ferritin levels below 30 ng/l and serum iron levels below 65/50 µg/dl in males and females, respectively, which reduced following a short iron treatment (Keser *et al.,* 2021), and molecular testing of alpha thalassemia mutation by multiplex PCR. The samples were sent to Alborg Laboratory to confirm the diagnosis of α-thalassemia through molecular testing.

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serum iron levels below 65/50 µg/dl in males and females, respectively, which reduced following a short iron treatment. (Yonder & Pandey, 2024).

Haematological Analysis:

To get the parameters of whole blood samples (MCH, MCV and MCHC), the Sysmex XN-1000 automated haematology analyzer (Sysmex America Inc., Lincolnshire, IL, USA) was utilized.

Hematological parameters were recorded on a standard blood cell counter. Patients were identified with microcytic hypochromic red blood cells (RBCs). Hb thresholds were according to those established by the World Health Organization (2008). HB electrophoresis was done by capillary electrophoresis.

Molecular Screening:

Molecular screening was performed using a reverse-hybridization assay) based on multiplex-PCR (Alpha-Globin StripAssay®) (Vienna Lab Diagnostics Vienna, Austria) (Puehringer et al., 2007), (13) .specific for α2 poly A2, alpha 2 poly1, α2 IVS1 and $--^{MED}$ gene mutations for the rapid and simultaneous detection of alpha-globin mutations.

We used a commercial approach (Alpha-globin StripAssay; Vienna Lab Diagnostics, Vienna, Austria) based on reverse dot blot hybridization (RDBH) and multiplex-PCR to screen the samples for the 21 known mutations of the alpha globin genes. There are 21 different α-globin mutations that the test covers: 3.7 single gene deletion, 4.2 single gene deletion, MED double gene deletion, SEA double gene deletion, THAI double gene deletion, FIL double gene deletion, 20.5 kb double gene deletion, anti-3.7 gene triplication, α1 cd 14 [TGG>TAG], α1 cd 59 [GGC>GAC] (Hb Adana), α2 init cd [ATG>ACG], α2 cd 19 [-G], α2 IVS1 [-5nt], α2 cd 59 [GGC>GAC], α2 cd 125 [CTG>CCG] (Hb Quong Sze), α2 cd 142 [TAA>CAA] (Hb Constant Spring), α2 cd 142 [TAA>AAA] (Hb Icaria), α2 cd 142 [TAA>TAT] (Hb Pakse), α2 cd 142 [TAA>TCA] (Hb Koya Dora), α2 poly A-2 [AATAAA-AATGAA] and α2 poly A-1 [AATAAA-AATAAG],

The process had three stages: First; isolation of DNA followed by PCR amplification using biotinylated primers. Lastly, the process of amplification product hybridization with test strips that have an array of parallel lines immobilized with allele-specific oligonucleotide probes. Using streptavidin-alkaline phosphatase and color substrates, bound biotinylated sequences were identified.

The frequencies of the alleles were calculated after various α-globin genotypes were identified.

Statistical Methods:

The collected data was analysed using appropriate statistical tests of Statistical Package for Social Science (IBM Corp. Released in 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.). With the use of the Student T Test, we determined if the difference between the two study group means was statistically significant. For determining if a non-parametric variable differed significantly between the two research groups, the Mann Whitney Test (U test) was employed. The correlation between the two qualitative variables was studied using a chi-square test. A statistically significant result was defined as a P-value < 0.05, and all tests were two-sided.

RESULTS

Testing Data Normality:

Data of age, RBCs, MCV, MCH, platelets, and RDW are significantly different from the normal distribution. Data of Hb is normally distributed (p value of Kolmogorov-Smirnov is more than 0.05) as shown in Table (1).

Table 1: Testing data normality

Parameter	P value
age	<.001
Hb	.070
MCV	.019
MCH	<.001
PLAT	<.001
RDW	<.001
age	<.001
Hb	.070
MCV	.019

Subject Demographics And Clinical Features:

The present study was conducted on 200 α -thalassemia cases. Their median age was 24.4, ranging from 1 to 60 years. They were 50.5% males and 49.5% females, as shown in Table 2. The mean Hb was 11.746 g/dL, the mean RBC was 5.57, the mean MCV was 71.8, the mean MCH was 21.418, the mean RDW was 17.72, the median platelet count was $329.5X10^9$/L (Table 2).

Table 2: Baseline features of all studied cases**.**

		α -thalassemia	
		N=200	
Male	**N (%)**	101	(50.5%)
female	**N (%)**	99	(49.5%)
Age	**mean±SD Median(range)**	24.41±8.515	25(1-60)
Hb	**mean±SD; Median(range)**	11.746 ±2.0512	11.8 (6-11)
RBC	**mean±SD; Median(range)**	5.57±0.76	5.59(3.45-7.7)
MCV	**mean±SD; Median(range)**	71.8±9.21	72(24-100)
MCH	**mean±SD; Median(range)**	21.42±3.17	22(12-35)
RDW	**mean±SD; Median(range)**	17.72±4	17(12-35)
Platelet	**mean±SD; Median(range)**	371.21±160.3	329.5(150-1598)

In our study, seventeen different alpha-globin gene mutations were determined in 200 patients. They were classified into 3 categories: deletional, non-deletional, and compound mutations (Table 3).

Table 3: Genes affected among α-thalassemia cases.

Type of α- Globin Genotype	Frequency	Percentage
Deletional	**108**	**54**
3.7 homo ($-\alpha^{3.7}/-\alpha^{3.7}$)	69	39
3.7 hetero ($-\alpha^{3.7}/\alpha\alpha$)	37	18.5
Alpha 4.2 hetero ($-\alpha^{4.2}/\alpha\alpha$)	1	.5
AA Med hetero ($--^{MED}/\alpha\alpha$)	1	.5
Non Deletional	**45**	**22.5**
ALPHA 2 POLYA1 homozygous (α2polyA1/α2polyA1)	16	8.0
Anti 3.7 heterozygous ($anti^{3.7}/\alpha2$)	2	1.0
Alpha 2 IVS1 homozygous (α2IVS1/ α2IVS1)	2	1.0
Alpha2 poly A1-heterozygous (α2polyA1/α2)	1	.5
Alpha 2 IVS1 hetero (α2IVS1/α2)	19	9.5
Alpha2 polyA2 heterozygous (α2 polyA2/ α2)	5	2.5
Compound	**48**	**24**
3.7 hetero and poly A hemizygous ($\alpha^{3.7}\alpha\alpha$ /polyA)	32	16
Alpha2 IVS1 hetero and polyA1 heterozygous (α2IVS1α2/ polyA1 α2)	3	1.5
3.7 hetero and alpha2 IVS I hemizygous ($\alpha^{3.7}\alpha$ α / α2 IVS1)	8	4
4.2 hetero and alpha 2 poly A1 hemizygous ($\alpha^{4.2}$ α / α2 poly A1)	1	.5
3.7 homo and 4.2 homozygous ($\alpha^{3.7}$ $\alpha^{3.7}/\alpha^{4.2}$ $\alpha^{4.2}$)	1	.5
Med double hetero and alpha2 polyA2 hetero ($--^{MED}$ αα /α2polyA2)	2	1
Alpha 2 IVS I hetero and alpha 2 poly A2 heterozygous (α2IVS Iαα /α2 polyA2 αα)	1	.5
Total	**200**	**100**

No significant associations were found regarding gender with alpha thalassemia genotype distribution (p value=0.139) although the -$\alpha^{3.7}$/αα genotype was seen more frequently in females. and the α2polyA1/α2polyA1− genotype was more common in males (Table 4).

Table 4: Gender distribution among the studied gnotypes Genotype Gender Crosstabulation

Genotype	Gender			
	Male	**Female**	**Total**	
3.7 homo	34	35	69	
ALPHA 2 POLYA1 Homo	11	5	16	
3.7 hetero	15	22	37	
Alpha 2 IVS1 homo	0	2	2	
Alpha 2 IVS1 hetero	14	5	19	
3.7 hetero and poly A hemizygous	15	12	27	
Alpha2 IVS1 hetero and polyA1 hetero	1	1	2	
AlphaA2 3.7 hetero and poly A1 hemizygous	0	1	1	
AA Med hetero	0	1	1	
3.7 Alpha2 Polya1 Hemi	2	1	3	
3.7 homo and 4.2 homo	0	1	1	
Med double hetero and alpha2 polyA2 hetero	0	1	1	
Alpha2 polyA2 hetero	2	3	5	
Alpha2 polyA1 hemi and 3.7 hetero	1	0	1	
3.7 Alpha2 IVS1 Hemi	1	0	1	
3.7 hetero and IVS1 hemi	1	5	6	
Alpha2 polyA1 hetero and alpha2 IVS1 hetero	0	1	1	
3.7 hetero and alpha2 IVS1 homo	0	1	1	
Anti 3.7 hetero	1	1	2	
Alpha2 poly A1-hetero	1	0	1	
4.2 hetero + alpha2 polyA-1 hemi	1	0	1	
Alpha2 IVS1 hetero+alpha2 polyA2 hetero	0	1	1	
Alpha 4.2 hetero	1	0	1	
Total	101	99	200	
P value			0.139	

The first common abnormality in our study was (deletional) -$\alpha^{3.7}$/ -$\alpha^{3.7}$ in 69 patients with (34.5%), the second common also (deletional)is -$\alpha^{3.7}$/ α α in 37 patients with (18.5%), the third common abnormality is $\alpha^{3.7}$αα /α poly A genotype; 32 patients have this compound mutation (16%), the fourth common mutation is (non-deletional) α2IVS1/ α2 genotype with 19 patients (9.5%), the fifth common genotype is also (non-deletional) α2 polyA1/α2 polyA1 with 16 patients (8%), the sixth common mutation is a compound mutation of $\alpha^{3.7}$α α / α2 IVS1 gnotype; 8 patients with (4%), the seventh common mutation is (non-deletional) α2 polyA2/ α2; 5 patients (2.5%).

The less common genotypes in our study, which are of single mutations were 2 patients have anti$^{3.7}$/α2, 1 patient has α2 polyA1/α2, 1 patient has --^{MED} /αα, and also 1patient has -$\alpha^{4.2}$ / α α. genotype. As regards the rare compound mutations in our study, 3 patients have α2 IVS1 α2/ polyA1 α2, 2 patients have --MED αα /α2 poly A2 α2, and also 1 patient has α2 IVS I αα / α2 polyA2 αα, 1 patient has $\alpha^{4.2}$ α / α2 poly A1) genotype.

Haematological Parameters Analysis Among the studied Genotypes:

Lower hemoglobin level was significantly associated with the presence of --MED αα /α2polyA2α2 genotype (*p* value=0.027) and the absence of anti$^{3.7}$/α2 genotype (p value=0.009) among α-thalassemia cases. Otherwise, no significant association was found regarding MCV with other affected genes (Table 5).

Table 5: Haemoglobin among different mutations of α -thalassaemia patient**.**

Category	Genotype		Hb (g/dl) median & interquartile range	P value
Deletional (n=108)	$-\alpha^{3.7}/-\alpha^{3.7}$	Absent (N=131)	11.200(8.6-13.8)	0.241
		Present (N=69)	12.000(9.4-14.6)	
	$-\alpha^{3.7}/\alpha\alpha$	Absent (N=163)	11.4 (8.7-14.1)	0.206
		Present (N=37)	12.5 (10.8-14.2)	
	$-\alpha^{4.2}/\alpha\alpha$	Absent (199)	11.8(14.3-9.3)	0.75
		Present (1)	12.4	
	$--^{MED}/\alpha\alpha$	Absent (199)	11.8 (9.3-14.3)	0.905
		Present (1)	11.5	
Non deletional (n=45)	$anti^{3.7}/\alpha2$	Absent (N=198)	11.75 (9.25-14.25)	0.009
		Present (N=2)	15	
	α2polyA1/α2polyA1	Absent (N=184)	12.0 (9.3-14.7)	0.201
		Present (N=16)	9.5 (6.9-12.1)	
	α2polyA1/α2	Absent (N=199)	11.8 (9.3-14.3)	0.512
		Present (N=1)	10.4	
	α2polyA2/ α2	Absent (N=195)	11.8(9.3-14.3)	0.557
		Present (N=5)	(8.9-15.9)12.4	
	α2IVS1/α2IVS1	Absent (N=198)	11.8 (9.2-14.4)	0.409
		Present (N=2)	10.55	
	α2IVS1/α2	Absent (N=181)	11.8 (9.2-14.4)	0.451
		Present (N=19)	11.5 (8.5-14.5)	
Compound (n=48)	$\alpha^{3.7}\alpha$ α / α2 IVS1	Absent (N=192)	11.775	0.751
		Present (N=8)	11.050	
	$\alpha^{3.7}\alpha\alpha$ /polyA	**Absent (N=168)**	11.800 (9.3-14.3)	0.52
		Present (N=32)	10.500 (8.1-12.6)	
	$\alpha^{3.7}\ \alpha^{3.7}/\alpha^{4.2}\ \alpha^{4.2}$	Absent (N=199)	11.8 (9.3-14.2)	0.319
		Present (N=1)	9.7	
	$--^{MED}\ \alpha\alpha$ /α2polyA2	Absent (N=198)	11.8(9.3-14.3)	0.027
		Present (N=2)	7.9	
	$\alpha^{4.2}$ α / α2 poly A1	Absent (N=199)	11.8 (9.3-14.3)	0.610
		Present (N=1)	10.7	
	α2IVS1α2/ polyA1 α2	Absent (N=197)	11.8 (9.3-14.3)	0.389
		Present (N=3)	9	
	α2IVS Iαα /α2 polyA2 αα	Absent (N=199)	11.8 (9.3-14.3)	0.790
		Present (N=1)	10.6	

Lower RBC count was significantly associated with the presence of --MED αα /α2 poly A2α2 genotype (*p* value= 0.042) among α-thalassemia cases. Otherwise, no significant association was found regarding RBCs count with other affected genes (Table 6).

Table 6: RBCs among different mutations of α -thalassaemia patient**.**

Category	Genotype		RBCs median & interquartile range	P value
Deletional (n=108)	-$\alpha^{3.7}$/ -$\alpha^{3.7}$	Absent (N=131)	5.5800 (4.66-6.5)	.836
		Present (N=69)	5.60 (4.84-6.36)	
	-$\alpha^{3.7}$/αα	Absent (N=163)	5.6 (4.7-6.5)	.298
		Present (N=37)	5.3550 (4.425-6.285)	
	-$\alpha^{4.2}$ /αα	Absent (199)	5.58(4.67-6.48)	0.660
		Present (1)	5.86	
	--^{MED}/αα	Absent (199)	5.5900(4.68-6.5)	0.890
		Present (1)	5.5	
Non deletional (n=45)	anti$^{3.7}$/α2	)198(N= Absent	5,58 (4.67-6,48)	.477
		Present (N=2)	6.455	
	α2polyA1/α2polyA1	84)1(N= Absent	5.58 (4.67-6.49)	0.765
		Present (N=16)	5.8(4.38-7.22)	
	α2polyA1/α2	Absent (N=199)	.58(4.67-6.48)5	0.636
		Present (N=1)	5.26	
	α2polyA2/ α2	Absent (N=195)	5.5800 (4.68-6.48)	.636
		Present (N=5)	6.4400 (3.79-9.09)	
	α2IVS1/α2IVS1	Absent (N=198)	5.595 (4.695-6.495)	0.465
		Present (N=2)	5.29	
	α2IVS1/α2	Absent (N=181)	5.5800 (4.67-6.49)	0.747
		Present (N=19)	5.7000 (4.7-6.7)	
Compound (n=48)	$\alpha^{3.7}$α α / α2 IVS1	Absent (N=192)	5.5750 (4.66-6.5)	.718
		Present (N=8)	5.6000 (5.15-6.05)	
	$\alpha^{3.7}$αα /polyA	Absent (N=	5.5900 (4.67-6.51)	0.220
		Present (N=32)	5.6000 (4.69-6.51)	
	$\alpha^{3.7}$ $\alpha^{3.7}$/$\alpha^{4.2}$ $\alpha^{4.2}$	Absent (N=199)	5.5900 (4.68-6.5)	0.790
		Present (N=1)	5.4	
	--^{MED} αα /α2polyA2	Absent (N=198)	5.5900 (4.69-6.49)	0.042
		Present (N=2)	4.8	
	$\alpha^{4.2}$ α / α2 poly A1	Absent (N=199)	5.58(4.67-6.48)	0.667
		Present (N=1)	5.83	
	α2IVS1α2/ polyA1 α2	Absent (N=197)	.58(4.67-6.48)5	0.477
		Present (N=3)	5.300	
	α2IVS Iαα /α2 polyA2 αα	Absent (N=199)	5.5800 (4.67-6.49)	0.220
		Present (N=1)	5.6000	

Lower MCV was significantly associated with the presence of α2 poly A1/α2 polyA1 among α-thalassemia cases (*p* value <0.001). Otherwise, no significant association was found regarding MCV with other affected genes (Table 7).

Table 7: MCV among different mutations of α -thalassaemia patient**.**

Category	Genotype		MCV median & interquartile range	P value
Deletional (n=108)	$-\alpha^{3.7}/-\alpha^{3.7}$	Absent (N=131)	71.0 (57-85)	.836
		Present (N=69)	73(65-81)	
	$-\alpha^{3.7}/\alpha\alpha$	Absent (N=163)	70.250 (60.3-80.15)	.298
		Present (N=37)	80 (75-85)	
	$-\alpha^{4.2}/\alpha\alpha$	Absent (199)	(61-83)72	.936
		Present (1)	71	
	$--^{MED}/\alpha\alpha$	Absent (199)	72 (61-83)	0.440
		Present (1)	66	
Non deletional (n=45)	$anti^{3.7}/\alpha2$	)198(N= Absent	(61-83)72	.549
		Present (N=2)	72	
	α2polyA1/α2polyA1	84)1(N= Absent	72 (60.5-83.5)	<0.001
		Present (N=16)	65 (50.8-79.2)	
	α2polyA1/α2	Absent (N=199)	(61-83)72	0.440
		Present (N=1)	66	
	α2polyA2/ α2	Absent (N=195)	72 (61-83)	.440
		Present (N=5)	75 (57.7-92.3)	
	α2IVS1/α2IVS1	Absent (N=198)	72 (61-83)	0.134
		Present (N=2)	65	
	α2IVS1/α2	Absent (N=181)	71 (59.7-82.3)	0.069
		Present (N=19)	77 (69-85)	
Compound (n=48)	$\alpha^{3.7}\alpha$ α / α2 IVS1	Absent (N=192)	72 (61-83)	.114
		Present (N=8)	66.5 (60.2-72.8)	
	$\alpha^{3.7}\alpha\alpha$ /polyA	Absent (N=	72 (61-83)	0.12
		Present (N=32)	67 (59-80)	
	$\alpha^{3.7}$ $\alpha^{3.7}/\alpha^{4.2}$ $\alpha^{4.2}$	Absent (N=199)	(61-83)72	0.936
		Present (N=1)	70	
	$--^{MED}$ αα /α2polyA2	Absent (N=198)	72 (61-83)	0.684
		Present (N=2)	72	
	$\alpha^{4.2}$ α / α2 poly A1	Absent (N=199)	72 (61-83)	.936
		Present (N=1)	67	
	α2IVS1α2/ polyA1 α2	Absent (N=197)	72 (61-83)	.549
		Present (N=3)	69.5	
	α2IVS Iαα /α2 polyA2 αα	Absent (N=199)	72 (61-83)	0.684
		Present (N=1)	72	

Lower MCH was significantly associated with the absence of $-\alpha^{3.7}/\alpha\alpha$ (*p* value <0.001), α2IVS1/α2 (p value= 0.004) and $--^{MED}/\alpha\alpha/\alpha2$ poly A2α2 (*p* value=0.044), presence of α2polyA1/α2polyA1 (*p* value <0.001) and $\alpha^{3.7}\alpha\alpha/\alpha2$ IVS1 (*p* value=0.047), among α-thalassemia cases. Otherwise, no significant association was found regarding MCH with other affected genes (Table 8).

Table 8: Association of MCH with affected gene among α-thalassemia cases.

Category	Genotype		MCH median & interquartile range	P value
Deletional (n=108)	$-\alpha^{3.7}/-\alpha^{3.7}$	Absent (N=131)	21(14-27)	.120
		Present (N=69)	22 (20.4-23.6)	
	$-\alpha^{3.7}/\alpha\alpha$	Absent (N=163)	21(17.4-24.6)	<.001
		Present (N=37)	24 (22-26)	
	$-\alpha^{4.2}/\alpha\alpha$	Absent (199)	(17.4-26.6)22	.838
		Present (1)	21	
	$--^{MED}/\alpha\alpha$	Absent (199)	22 (17.4-26.6)	0.838
		Present (1)	21	
Non deletional (n=45)	$anti^{3.7}/\alpha2$	)198(N= Absent	22 (17.4-26.6)	.663
		Present (N=2)	22	
	α2polyA1/α2polyA1	84)1(N= Absent	22 (18-26)	<0.001
		Present (N=16)	17 (15-19)	
	α2polyA1/α2	Absent	22 (17.4-26.6)	.047
		Present	19.5 (17-22)	
	α2polyA2/ α2	Absent (N=199)	22 (18-26)	.838
		Present (N=1)	22	
	α2IVS1/α2IVS1	Absent (N=195)	22 (17.4-26.6)	0.101
		Present (N=5)	23 (18.2-27.8)	
	α2IVS1/α2	Absent (N=198)	22 (17.3-26.7)	0.663
		Present (N=2)	20	
	$\alpha^{3.7}\alpha\ \alpha$ / α2 IVS1	Absent (N=181)	21(17-25)	0.004
		Present (N=19)	23 (20-26)	
Compound (n=48)	$\alpha^{3.7}\alpha\ \alpha$ / α2 IVS1	Absent (N=192)	22 (17.4-26.6)	.047
		Present (N=8)	19.5 (17-22)	
	$\alpha^{3.7}\alpha\alpha$ /polyA	Absent (N=	22 (17.4-26.6)	.838
		Present (N=32)	19 (17-21)	
	$\alpha^{3.7}\ \alpha^{3.7}/\alpha^{4.2}\ \alpha^{4.2}$	Absent (N=199)	22 (17.4-26.6)	0.650
		Present (N=1)	20	
	$--^{MED}\ \alpha\alpha$ /α2polyA2	Absent (N=198)	22 (17.4-26.6)	0.044
		Present (N=2)	28	
	$\alpha^{4.2}\ \alpha$ / α2 poly A1	Absent (N=199)	22 (17.4-26.6)	.838
		Present (N=1)	18.5	
	α2IVS1α2/ polyA1 α2	Absent (N=197)	22 (17.4-26.6)	.663
		Present (N=3)	19.8	
	α2IVS Iαα /α2 polyA2 αα	Absent (N=199)	22 (17.4-26.6)	.838
		Present (N=1)	17.5	

Higher RDW was significantly associated with the presence of α2polyA1/α2 polyA1, $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{3.7}/-\alpha^{3.7}$ and α2IVS1/α2 among α-thalassemia cases. Otherwise, no significant association was found regarding RDW with other affected genes (Table 9).

Table 9: RDW among different mutations of α -thalassaemia patient**.**

Category	Genotype		RDW median & interquartile range	P value
Deletional (n=108)	$-\alpha^{3.7}/-\alpha^{3.7}$	Absent (N=131)	17.20 (12.2-22.2)	.041
		Present (N=69)	17 (14.5-19.5)	
	$-\alpha^{3.7}/\alpha\alpha$	Absent (N=163)	17 (13.4-20.6)	<.001
		Present (N=37)	15 (12-18)	
	$-\alpha^{4.2}/\alpha\alpha$	Absent (199)	(13-21)17	.290
		Present (1)	21	
	$--^{MED}/\alpha\alpha$	Absent (199)	17 (13-21)	0.530
		Present (1)	15	
Non deletional (n=45)	$anti^{3.7}/\alpha2$	)198(N= Absent	(13-21)17	.334
		Present (N=2)	17	
	α2polyA1/α2polyA1	84)1(N= Absent	17 (13.5-21.5)	<0.001
		Present (N=16)	25 (21-29)	
	α2polyA1/α2	Absent (N=199)	(13-21)17	.863
		Present (N=1)	17	
	α2polyA2/ α2	Absent (N=195)	17 (13-21)	0.855
		Present (N=5)	17 (13-20)	
	α2IVS1/α2IVS1	Absent (N=198)	(13-21)17	0.334
		Present (N=2)	18.5 (0)	
	α2IVS1/α2	Absent (N=181)	17(13-21)	0.032
		Present (N=19)	15 (12-18)	
Compound (n=48)	$\alpha^{3.7}\alpha$ α / α2 IVS1	Absent (N=192)	17 (13-20)	0.100
		Present (N=8)	18.7 (16.2-21.2)	
	$\alpha^{3.7}\alpha\alpha$ /polyA	Absent (N=	17(13-21)	.361
		Present (N=32)	18.2 (15.2-21.2)	
	$\alpha^{3.7}$ $\alpha^{3.7}/\alpha^{4.2}$ $\alpha^{4.2}$	Absent (N=199)	(13-21)17	0.510
		Present (N=1)	19	
	$--^{MED}$ αα /α2polyA2	Absent (N=198)	(13-21)17	0.90
		Present (N=2)	17	
	$\alpha^{4.2}$ α / α2 poly A1	Absent (N=199)	(13-21)17	.863
		Present (N=1)	17	
	α2IVS1α2/ polyA1 α2	Absent (N=197)	(13-21)17	.334
		Present (N=3)	17.25	
	α2IVS Iαα /α2 polyA2 αα	Absent (N=199)	(13-21)17	.863
		Present (N=1)	17	

Higher platelet count was significantly associated with the presence of $--^{MED}$ αα /α2polyA2 genotype (*p* value= 0.030) and α2polyA1/α2polyA1 genotype (*p* value <0.001). Otherwise, no significant association was found regarding platelet count with other affected genes (Table 10).

Table 10: Association of platelet count with affected gene among α-thalassemia cases.

Category	Genotype		Platelets median & interquartile range	P value
Deletional (n=108)	$-\alpha^{3.7}/-\alpha^{3.7}$	Absent (N=131)	334 (151-517)	.408
		Present (N=69)	320 (187-453)	
	$-\alpha^{3.7}/\alpha\alpha$	Absent (N=163)	326.5 (154.5-498.5	.916
		Present (N=37)	332 (209-455)	
	$-\alpha^{4.2}/\alpha\alpha$	Absent (199)	(176-482)329	0.100
		Present (1)	196	
	$--^{MED}/\alpha\alpha$	Absent (199)	330 (169-491)	0.079
		Present (1)	282	
Non deletional (n=45)	$anti^{3.7}/\alpha2$	)198(N= Absent	(176-482)329	0. .477
		Present (N=2)	365	
	α2polyA1/α2polyA1	84)1(N= Absent	324 (180-468)	<0.001
		Present (N=16)	520 (212-828)	
	α2polyA1/α2	Absent (N=199)	(176-482)329	1.000
		Present (N=1)	329	
	α2polyA2/ α2	Absent (N=195)	329 (176-482)	0.692
		Present (N=5)	392 (150-634)	
	α2IVS1/α2IVS1	Absent (N=198)	331.50 (168.5-494.5)	0.477
		Present (N=2)	302	
	α2IVS1/α2	Absent (N=181)	330 (164-496)	0.910
		Present (N=19)	325 (189-461)	
Compound (n=48)	$\alpha^{3.7}\alpha$ α / α2 IVS1	Absent (N=192)	328.5 (167.5-489.5)	.718
		Present (N=8)	394 (240-548)	
	$\alpha^{3.7}\alpha\alpha$ /polyA	Absent (N=	329 (174-484)	0.633
		Present (N=32)	344 (80-608)	
	$\alpha^{3.7}$ $\alpha^{3.7}/\alpha^{4.2}$ $\alpha^{4.2}$	Absent (N=199)	329 (168-497)	0.870
		Present (N=1)	351	
	$--^{MED}$ αα /α2polyA2	Absent (N=198)	329 (176-482)	0.030
		Present (N=2)	852	
	$\alpha^{4.2}$ α / α2 poly A1	Absent (N=199)	176-482)329	0.670
		Present (N=1)	298	
	α2IVS1α2/ polyA1 α2	Absent (N=197)	330 (174-486)	.477
		Present (N=3)	213	
	α2IVS Iαα /α2 polyA2 αα	Absent (N=199)	(176-482)329	0.950
		Present (N=1)	232	

Two patients had $anti^{3.7}/\alpha2$ they had no anemia with a median HB of 15 and MCV 72, MCH 22, RBCs 6.45. They have good compensatory erythrocytosis.Only one patient has α2polyA1/α2 and a median HB of 10.4, RBCs of 5.26, MCV of 66, and MCH of 22.

DISCUSSION

One in five people on Earth has the alpha thalassemia gene, according to the World Health Organization (WHO) (Modell & Darlison, 2008). Alpha thalassemia is more common in regions of the Middle East and the Mediterranean. Approximately 40% more carriers are present in that area (Vichinsky, 2013).

Genetic counseling and choosing patients at high risk for disease are both aided by the molecular diagnosis of -thalassemia. Thalassemia is a prevalent hereditary disease in Saudi Arabia (Olwi *et al.*, 2018). Haematological investigation of common genotypes and mapping of high-incidence regions should be ongoing priorities.

Gender is becoming more important in the medical industry since it can impact disease prognosis (Kyriakou *et al.*, 2008). No significant associations were found regarding gender with alpha thalassemia genotype

distribution, although the -α$^{3.7}$/αα genotype was more common in females and α2polyA1/α2polyA1 genotype was more common in males. This finding is consistent with the previous studies, which demonstrated that -α$^{3.7}$/αα genotype was more common in females (Alhuthali *et al.*, 2023), (Anselmo et al., 2019). The frequency of thalassemia carriers was found to be significantly higher in females than in males, according to Husna and Sanka (Husna & Handayani, 2021). Moreover, a different study that looked at gender in non-transfusion-dependent thalassemia patients found that women were more likely to be anaemic than men. It may be necessary to follow up with pregnant women who are thalassemia carriers or have features and offer good genetic counseling, as there were no significant differences regarding the illness complication (Marsella et al., 2018).

The -α$^{3.7}$/-α$^{3.7}$ genotype was the most frequent genotype followed by -α$^{3.7}$/αα and α$^{3.7}$αα /polyA genotype respectively. The less common genotypes in our study are of single mutations were 2 patients have anti$^{3.7}$/α2 genotype,-α2 polyA1/α2, --^{MED} /αα, and -α$^{4.2}$ / α α. genotype. Our results are in accordance with Keser et al and Alhuthali et al (Keser et al., 2021) (Alhuthali et al., 2023). whom denoted that -α3.7 deletion mutations is the most common mutation in Saudi Arabia. Similar distributions of mutations have been reported in other countries, including Malaysia (Vijian D et al., 2023), Cambodia (Munkongdee et al., 2016), and Laos (Wongprachum *et al.*, 2016).

Overall, half of the participants in the present study had deletional mutations (54.5%), followed by compound mutations (24%), and finally non-deletion mutations (22.5%). This is in accordance with Alhuthali *et al.* (Alhuthali *et al.*, 2023).Our results are in disagreement with Hellani and collaegues study that denoted that Point mutations have been reported to be more prevalent than large deletions among Arabs (Hellani et al., 2009). This discrepancy may be attributed to different sample sizes and geographical distribution.

Lower hemoglobin level was significantly associated with presence of–--^{MED}αα/α2polyA2 and absence of anti$^{3.7}$/α2 genotype among α-thalassemia cases. Otherwise, no significant association was found regarding MCV with other affected genes. Our findings are consistent with Akhavan-Niaki et al who denoted that Individuals with a single mutant gene typically exhibit normal hemoglobin levels, whereas individuals with two defective genes (α-thalassemia trait) display significantly diminished hemoglobin levels, resulting in mild anemia. (Akhavan-Niaki *et al.*, 2012).

Lower RBC count was significantly associated with presence of --^{MED}αα/α2polyA2 genotype among α-thalassemia cases. Otherwise, no significant association was found regarding RBCs count with other affected genes. Lower MCV was significantly associated with presence of α2polyA1/α2polyA1 among α-thalassemia cases . Otherwise, no significant association was found regarding MCV with other affected genes. Our results are in disagreement with Vijian et al study that showed significant changes in RBCs and Hb among patients with deletional mutations (Vijian D et al., 2023). This discrepancy is attributed to the fact that It would be impossible to characterize every thalassemia mutation using only haematological measures and it is common for the haematological parameters to vary even within the same genotype (Vijian D et al., 2023). Variations could be influenced by factors in the environment and genes.

Lower MCH was significantly associated with absence of -α$^{3.7}$/αα (p value <0.001), α2IVS1/α2 (p value= 0.004) and --^{MED} αα /α2 poly A2 and presence of α2polyA1/α2polyA1 and 3.7 α$^{3.7}$α α / α2 IVS1 genotype (*p* value=0.047), among α-thalassemia cases. Otherwise, no significant association was found regarding MCH with other affected genes. Our findings are consistent with prior studies that mentioned that patients who had two functional alpha globin genes had lower MCV and MCH than those who had one mutant gene. (Rizo-de la Torre *et al.*, 2021). Additionally, regardless

of the sample size, the MCH data consistently and significantly differs among different genotypes (Husna & Handayani, 2021).

The results of this research could be used to improve public health services in Middle East by informing guidelines for the identification and treatment of thalassemia. It proposes that it is necessary to enhance health awareness and education initiatives primarily in places with a higher risk to expand the population's understanding about the disease and repercussions of consanguineous marriages. If we want to significantly reduce the prevalence of this sickness, we need to ensure that at-risk couples are fully informed about the severity of the disease before they decide to get married through a more effective genetic counseling program. To confirm these results and include other prevalent mutations, it is recommended that additional research be conducted in a bigger population and on a larger scale to cover all genes that potentially contribute to the illness occurrence (Alhuthali *et al.*, 2023).

The strength of our study is the proven reliability of The Alpha-Globin Strip Assay as a screening method to identify >90% of α-globin mutations in endemic areas all over the globe (Puehringer *et al.*, 2007). Moreover, the power of the sample size of this study is adequate increasing the accuracy of our results.

There are several constraints that need to be appointed in this study. To begin, because it's an analysis of past data, the data are restricted to what was available in the laboratory at the time. For instance, prior research into the genetic basis of alpha-thalassemia was limited to the diagnostic assay proven in the Alborg Diagnostic Lab, which evaluates only the 21 mutations of alpha globin genes. Second, there was a lack of confirmation of genetic mutations by DNA sequencing. The insufficient laboratory data additionally hindered the evaluation of haematological indices. More than that, we assume that most of the participants in this study comprised married couples, as the data came from a private diagnostic laboratory; as a result, we expect that the majority of the cases in our study were -thalassemia characteristics or carriers. Therefore, the prevalence of -thalassemia in Saudi Arabia may have been understated in the study. Lastly, the prevalence of -thalassemia genotypes in rural regions may be underestimated because Alborg Diagnostic Laboratory has locations in almost every one of Saudi Arabia's big cities. So, the results of this study cannot be generalized.

We suggest conducting additional research with a bigger sample size using molecular genetic methods such as Multiplex Ligation-dependent Probe Amplification (MLPA) and DNA sequencing. We recommend that in the therapy of hemoglobinopathy, analytical methods such as carrier detection, prenatal testing, and the provision of genetic information during the premarital period be planned. Screening for alpha thalassemia mutations utilizing complementary molecular genetic approaches in specific locations is clinically and technically necessary.

Conclusion:

The study found that the most prevalent alpha thalassaemia abnormality is -$\alpha^{3.7}$/ -$\alpha^{3.7}$ genotype. Middle Eastern populations have high levels of variability and numerous origins mixing, as shown by the alleles observed. Variations in patients' Hb, RBCs, platelet count, RDW, MCH, and MCV levels were seen, even among those with the same genotypes. These differences may be the result of environmental and genetic modifiers. Because of this, hematological criteria alone cannot be used to uniquely characterize any alpha thalassaemia mutation.

Declarations

Ethics Approval and Consent to Participate: The research was approved by Al Borg Diagnostic Lab's biomedical ethics unit (10-2022). Due to the retrospective study design, the need for informed consent from all subjects or their parents/legal guardians was waived by Al Borg Diagnostic Lab's biomedical ethics unit.

Permission to Publish: Not relevant. The paper does not contain any personal information.

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Authors' Contributions: Conceptualization, D.E.A. and H.M.E; methodology, S.A., O.E. and A.F.G; software, S.A; validation, A.F.G; formal analysis, M.H.M; resources, D.E.A; data curation, H.M.E; writing—original draft preparation, M.H.M; writing—review and editing, D.E.A and M.H.M; The published version of the work has been reviewed and approved by all authors.
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