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Characterization of the Molecular Spectrum of α-Thalassemia Mutations in the Western Province of Saudi Arabia and Recommendation for Premarital Screening

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Abstract

Background: α-thalassemia trait is frequently encountered in Saudi Arabia with a large diversity and geographical variability in the underlying genotypes.

Aim of Study: To characterize the molecular spectrum of α-thalassemia in the western province of Saudi Arabia in some Saudi patients suspected of α thalassemia carrier or diagnosed with Hb H disease to determine if there is the significance of premarital genetic testing for α thalassemia in a couple suspected with α thalassemia trait.

Patients and Methods: This study included 39 patients, 34 patients with suspected α thalassemia trait and 5 patients diagnosed with Hb H disease.

Results: Thirteen patients 33.3% are heterozygous for + thalassemia having the genotype $--^{3.7}/$ and 14 patients 35.9% are homozygotes for 0 having the genotype $-3^{3.7}/-\alpha 3.7$ and, one patient with $--^{MED}/\alpha\alpha$, one with $--^{SEA}/\alpha\alpha$, and one $^{PA-1}$ / and the 5 patients with HGB H disease 12.8%, one patient has a genotype of $--^{SEA}/-^{3.7}$, one with $--^{MED}/-^{3.7}$ Mediterranean thalassemia (--MED) with 3.7 kb heterozygous deletion, and 2 patients with genotype $\alpha^{PA-1}/\alpha^{PA-1}$ homozygous mutation Poly A (A->G). There was one case with negative molecular screening for α-thalassemia.

Conclusion: The $-\alpha^{3.7}$ was the most common mutation among patients with α-thalassemia forming 78.9% of all deletions. The premarital genetic diagnosis of α-thalassemia is not recommended as $-\alpha^{3.7}$ deletion is not risk for hydrops fetalis but should be considered in families with a history of HGB H disease or hydrops fetalis.

Key ***Words:*** *a thalassemia – Hb H disease – Saudi Arabia.*

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Introduction

THE α-thalassemia trait exists at a high prevalence in Saudi Arabia, in western region about 40% while HGB H disease about 0.19% [1]. α-thalassemia is caused by impairment in α-globin chain production, which is normally regulated by 4 genes on 2 loci (α1 and α2). It is divided into 4 clinical subtypes according to the extent of α-globin chain deficiency. People with a single α-globin gene deletion (–α/αα) are described as silent α-thalassemia carriers while deletion of 2 α-globin genes (--/αα) or (-α/-α) are described as α-thalassemia trait. Mutations involving 3 α-globin genes (--/-α), give rise to Hb H disease. Deletion of all 4 alpha-genes (--/--) causes Hb Bart's hydrops fetalis, a condition that results in fetal death [2]. People with genotype α0-thalassemia (–/αα) or 3 genes deletion (--/-α), are carriers of Hb Bart hydrops fetalis. The size of the deletion in 0 alleles is variable and each is named for the part of the world where it is prevalent, thus –SEA, --FIL, –FIL, –MED are found in the South East Asia, Mediterranean, and Philippines respectively [3]. Population studies have indicated that the types and frequencies of the different α-thalassemia defects vary among different ethnic communities and tend to be geographically specific [4].

In Saudi Arabia the premarital screening program was initiated in 2004. The genetic diagnosis of α thalassemia trait is not included as the definite diagnosis of α thalassemia needs molecular tests and in addition the incidence of HGB H and hydrops fetalis disease are rare [5,6].

The main reason for genetic counseling for α-thalassemia syndromes is the avoidance of Hb Bart's Hydrops fetalis Syndrome. The HbH disease is usually mild (comparable to β-thalassemia intermedia), but the clinical and hematological severity can vary considerably. HbH disease is generally not considered for prenatal diagnosis unless a previous clinical history in the family has been recorded. The molecular knowledge about the mutations may allow some level of predictions regarding the expected disease severity and course this remains uncertain [7]. The polymerase chain reaction (PCR) is the most common method to diagnose deletional α-thalassemia, although other techniques such as Multiplex Ligation dependent Probe Amplification (MLPA) are widely used [8,9].

Patients and Methods

This study retrospectively analyzed the results of HPLC samples (32000) over a 8 years between February 2012 and February 2019. The study includeda total of 39 Saudi patients attending King Fahad Armed Forces Hospital Hematology Department, Jeddah, Saudi Arabia a major hospitals in the Western Province of Saudi Arabia. We found five patients diagnosed with Hb H disease and 34 patients were selected with possible thalassemia trait. All patients were subjected for CBC, HPLC, Hb H red cells inclusion body, serum iron, TIBC, %saturation, ferritin determination and α-globin chain genotyping. Selection of α-thalassemia trait was based on the patient with unexplained microcytichypochromic erythrocytes (mean corpuscular volume <80fL, and mean corpuscular hemoglobin <27pg), with increased red cell count with normal iron status and normal HPLC finding (Hb A2 below 3.5%) or being the available parent of a patient with Hb H disease or has Hb Bart's in newborn screening. The diagnosis of Hb H disease is based on the presence of Hemoglobin H peak fraction on HPLC of their RBC hemolysates supplemented by demonstration of Hemoglobin H inclusions with positive supravital stained peripheral blood smears [10,11].

Hematological analysis:

Following receipt of informed consent from all participants, blood samples (5ml) were collected in EDTA-coated vacutainers. Patients blood samples submitted for hemoglobinopathy diagnosis. A hematological parameters were analyzed with a Coulter automated hematology analyzer (Corporati, USA). Hb quantification was performed with VARIANTTM II Hemoglobin Testing System (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Specimen collection and the laboratory investigations:

Five milliliter overnight fasting blood samples were collected on both tri sodium EDTA (1mg/ml) tubes for subsequent genetic analysis study. The test was done in Uni. Lab. reference laboratory).

Molecule analysis: The assay covers 21 α-glob in mutations (Vienna Lab Labor diagnostic a GmbH, Vienna, Austria) [40]: 1-α 3.7 single gene deletion, 2-α 4.2 single gene deletion, 3-MED double gene deletion, 4-SEA double gene deletion, 5-THAI double gene deletion, 6- FIL double gene deletion, 7-20.5 kb double gene deletion, 8- anti-3.7 gene triplication, 9-α1 cd 14 (TGG>TAG), 10-α1 cd 59 [GGC>GAC] (Hb Adana), 11-α2 init cd [ATG>ACG] ,12-α2 cd 19 (-G), 13-α2 IVS1 (–5nt), 14-α2 cd 59 (GGC>GAC], 15-α2 cd 125 [CTG >CCG] (Hb Quong Sze), 16-α2 cd 142 [TAA >CAA) (Hb Constant Spring), 17-α2 cd 142 [TAA >AAA] (Hb Icaria), 18-α2 cd 142 [TAA>TAT] (Hb Pakse), 19-α2 cd 142 [TAA>TCA] (Hb Koya Dora), 20-α2 poly A-1 [AATAAA-AATAAG], 21-α2 poly A-2 [AATAAA-AATGAA]. Further genetic information is available at OMIM Online Mendelian Inheritance in Man: www.ncbi.nlm.nih.gov/omim. (Vienna Lab Labor diagnostic a GmbH, Vienna, Austria).

The procedure includes three steps: (1) DNA isolation and concentration (2) PCR amplification using biotinylated primers, (3) Hybridization of amplification products to test strips containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. α-Globin Strip Assay (catalog # 4-360, Vienna Lab, Austria) were used with the immobilized oligos on a test strip. Genotype interpretation was determined using the enclosed Collector TM sheet with certain scale. Positive and negative control lines were checked for strip validation; Positively stained lines were visually noted for each polymorphic position by two independent coauthors and signal pattern of bands were translated into schematic results using strip assay® online calculator [12].

Statistical methods: The collected data was analyzed using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Student *t*-Test was used to assess the statistical significance of the difference between two study group means. Mann Whitney Test (U test) was used to assess the statistical

significance of the difference of a non-parametric variable between two study groups. Chi-Square test was used to examine the relationship between two qualitative variables All tests were 2-sided and a p-value <0.05 was considered statistically significant.

Results

The present study was conducted on 39 α-thalassemia cases. Their median age was 5.0 years, ranged from 1.0 to 34 years. They were 26 (66.7%) males and 13 (33.3%) females. Mean Hb was 12.3g/dL, mean RBC was 5.6 x1012/l, mean MCV was 67 fl, mean MCH was 21.9pg as shown in Table (1). The HGB H disease cases were significantly associated with lower Hb, RBC, when compared to α-Thalathemia minor/trait cases while, there was non significant differences between both groups regarding age, gender, MCV, MCH as shown in Table (2).

Table (1): Baseline features of all studied cases.

		α-thalassemia cases N=39	
Age	Mean±SD; Median (range)	10.6±9.6	5 (1-34)
Male (N, %)		26	66.7%
Female (N, %)		13	33.3%
Hb g/dl	M±SD; Median (range)	12.3±1.6	12.3 (7.6-16.5)
RBC x1012/l	M±SD; Median (range)	5.6±0.5	5.6 (4.7-6.9)
MCV fl	M±SD; Median (range)	67±6.6	67 (54-81.1)
MCH pg	M±SD; Median (range)	21.9±2.5	22 (16.3-26)

Hb = Hemoglobin. MCV = Mean corpuscular volume.
RBCs = Red cell count. MCH = Mean corpuscular hemoglobin.

Table (2): Comparison of haematologic parameters between thalassemia minor / trait and HGB H disease groups.

		Thalassemia minor / trait N=34		HGB H N=5		*p*
Male	N, %	23	67.6%	3	60.0%	0.735
Female	N, %	11	32.4%	2	40.0%	
Age	M±SD, median (range)	10.9±11.2	5 (1-34)	8.2±5.8	8 (2-17)	0.998
HGB	M±SD, median (range)	12.7±1.3	12.4 (10.8-16.5)	10±1.4	10.8 (7.6-11)	0.001
RBCS	M±SD, median (range)	5.7±0.5	5.7 (4.9-6.9)	5.1±0.4	5.2 (4.7-5.5)	0.017
MCV	M±SD, median (range)	67.8±6.5	67 (56-81.1)	61.4±4.7	62 (54-67)	0.061
MCH	M±SD, median (range)	22.2±2.5	22 (17-26)	19.9±2.3	20 (16.3-22.6)	0.092

Our study estimates the type of α-thalassemia mutations genotype and allele frequencies in a total of 39 patients with α-thalassemia as introduced in Table (3). There was 13 patients with α-thalassemia minor (heterozygous α^+) (33.3%) have $-^{3.7}/\alpha\alpha$ genotype and 14 patients with α-thalassemia trait (35.9%) have compound homozygous-$^{3.7}/-\alpha^{3.7}$ genotype, and 4 patients have as follow $^{PA-1}$ / 1 (0.3%), 2 patients have --^{SEA} / αα (5.1%), one patient had --^{MED} /αα (2.6%), In addition, the 5 patients with Hb H disease (12.8%) showed one patient has compound heterozygous--^{MED}/-$^{3.7}$ genotype with a 17.5kb deletion which is associated with a Mediterranean thalassemia, the 2ed patient has compound heterozygous --^{SEA}/ $^{3.7}$ genotype with Southeast Asian --^{SEA} deletion, and 2 patients with compound homozygous mutation involving the α 2-globin gene with α^{PA-1}/ α^{PA-1} genotype with Poly A (A->G) often cited as $\alpha^{T-Saudi}$ α/$\alpha^{T-Saudi}$ α, and 1 patient have negative PCR test for mutations.

Table (3): Genotype and allele frequency of α-thalassemia mutations in 39 patients with α thalassemia trait and HbH disease.

	PCR	Chromosomes (78)		α-thalassemia N=39	
		Affected chromosome	Normal chromosome	N	%
α Thal minor/trait	-α3.7/ αα	13	13	13	33.3
	-α3.7/ -α3.7	28	0	14	35.9
	--MED / αα	1	1	1	2.6
	--SEA / αα	2	0	2	5.1
	α^{PA-1} / αα	4	4	4	10.3
HGB H	Negative	0	2	1	2.6
	--SEA/-α3.7	2	0	1	2.6
	--MED/-α3.7	2	0	1	2.6
	$\alpha^{PA-1}\alpha$ / $\alpha^{PA-1}\alpha$	4	0	2	5.1
	Total 5				12.8
Total		56	22	39	100

On estimation of total allele frequency of α-thalassemia mutations in the studied group. The 3.7 was the commonest mutation (78.9%) followed by α2 polyadenylation signal mutation (polyA1) (AATAAA>AATAAG ($\alpha^{PA-1}\alpha/\alpha^{PA1}\alpha$), being the most common (11.5%) in Hb H disease group, followed by --SEA (5.8%) and --MED (3.8%). In Hb H disease group the α^{PA-1} was the commonest one as in Table (4).

Table (4): The total allele frequency of α-thalassemia mutations in the studied groups.

α-thalassemia mutations	Total studied Chromosomes (78)	
	N. affected Chromosomes	%
$\alpha^{3.7}$	43	78.9
α^{PA-1}	8	11.5
--SEA	2	5.8
--MED	2	3.8
Total	56	100

Discussion

The α-thalassemia trait is frequently seen in several regions of Saudi Arabia. In the eastern region, the prevalence of α-thalassemia trait 28-60% has been reported in various reports **[13]**. It is possible to have patients with Hb H disease or Hydrops fetalis from a couple with α-thalassemia trait and this depends on the type of α thalassemia mutations. The molecular diagnosis of α-thalassemia is expensive and requires resources that are not available in many hospitals and at the same time, it is difficult to provide these molecular tests to all suspected cases of α thalassemia. Therefore the aim of this study is to identify the common α-thalassemia genotypes which will facilitate genetic counseling and the identification of patients who really deserve molecular diagnosis.

In this study, a total of 8 different genotypes combinations were detected in 39 patients with α thalassemia trait and Hb H disease. Throughout this study, we found that $-^{3.7}$ (78.9%), is the most common mutation in the total cases followed by α2 polyadenylation signal mutation (polyA1) (AATAAA>AATAAG ($\alpha^{PA1}\alpha/\alpha^{PA1}\alpha$), often cited as $\alpha^{T\text{-}Saudi}\alpha/\alpha^{T\text{-}Saudi}\alpha$ in 11.5%, then the --SEA (5.8%) and --MED (3.5%) while for a patient with Hb H disease group the ($\alpha^{PA1}\alpha/\alpha^{PA1}\alpha$) was the commonest one.

Our result is similar to Arwa et al. **[14]** who reported a total of eight genotype combinations were identified, with $\alpha^{PA1}\alpha/\alpha^{PA1}\alpha$, being the most common (53.8%) in Hb H disease followed by —MED/-3.7 (28.8%), also similar to Hellani et al. **[15]** who investigated 41 patients from the eastern province of Saudi Arabia with unexplained microcytic, hypochromic anemia, and found the prevalence of Rightward $-^{3.7}$ (64%), while polyA mutation showed a high prevalence (41%) so they recommended a strict molecular screening of all cases presenting with the unexplained hypochromic microcytic anemia. Similarly, Al-Awamy **[16]** revealed that in the Eastern region, 45% carried the (-3.7), while another 15% had a non-deletional defect (α-polyA1α).

The -3.7 is the most common α globin gene deletion. It is the 3.7 kb rightward deletion which is caused by the breakage of DNA molecules in

the α globin genes (HBA2 and HBA1) region and rejoining of the broken ends by leaving α globin genes region with a single functional gene. It is considered a benign mutation as it is always in transposition and can not produce α^0 thalassemia which usually cause Hb H disease or Hydropes fetalis. In our study, we found $-^{3.7}$ (78.9%), is the most common mutation and this is in agreement with Boegio et al. **[17]** who reported that the $-3^{.7}$ gene deletion is the most prevalent (43.5%) in the Saudi populations that were analyzed and are characterized by the deletion of 3,804 base pairs.

The same result also was present in countries in the Golf area as among the Kuwaitis with hemoglobin H disease, 17 patients 70.8 % had the ($\alpha^{PA^{-1}}\alpha/\alpha^{PA^{-1}}\alpha$), while 25% had the ($\alpha^{PA^{-1}}\alpha/-^{3.7}$) and 4.2 % were undetermined **[18]**. It is also common in Qatar as the $-^{3.7}$ deletion was the most common with 9.4% homozygotes, 30.0% heterozygotes, and an allele frequency of 19.7% in the Qatari Pediatric Population **[19]**. It has been also shown that Hb H disease is common in Bahrain (where people may belong to a common ethnic background to the east of Saudi Arabia) and is a result of homozygosity of non-deletion α-thalassemia $\alpha^{PA^1}\alpha/\alpha^{PA^1}\alpha$ (poly A signal mutation) **[20,21]**.

The α2-globin gene normally accounts for 2 to 3 times more α-globin mRNA and -globin chain production than the α1-globin gene **[22]**. The α-thalassemia point mutations of the α2-globin gene generally cause more severe anemia than the same mutations involving the α1-globin gene. In our study, a total of 2 patients were diagnosed with the genotype $\alpha^{PA^{-1}}\alpha/\alpha^{PA^{-1}}\alpha$. These homozygous patients presented with a typical form of Hb H disease and severe anemia. Second, we diagnosed 2 patients with the rightward ($-^{3.7}$) deletion genotype in combinations for $-3^{.7}/\text{--}^{SEA}$ and another one $-3.7/\text{--}^{MED1}$. These patients presented with a mild form of Hb H disease.

Hemoglobin H (Hb H) disease is less commonly seen in our western region of Saudi Arabia as over 8 years only 5 cases were diagnosed among 30200 HPLC test (0.01%).

The α^0 thalassemia (which is an important determinant to produce Hb H disease or Hb Bart's hydrops syndrome) is less frequently seen in the western region and in our study, only 9.2% of thalassemia mutations are α^0-thalassemia. While Hb H disease appears a frequently encountered disease in other areas in Saudi Arabia as in Dammam region where Quadri and Islam **[13]** found 100 cases of Hb H disease during 5 years among 15,492 blood samples subjected to Hb electrophoresis. This gives a laboratory-based incidence of 1 in 155 (0.64%).

The Southeast Asia deletion (--SEA) type has been found in 5.8% of our α-thalassemia mutations combined with $-^{3.7}$ and it is α^0-thalassemia deletion of approximately 19.3kb in length and removing both α-globin genes in cis but sparing the embryonic α-globin gene is common. This mutation is the most common cause of Hb H disease and hydrops fetalis syndrome in that part of the world in addition to the (--FIL), (--MED), and (α)20.5 deletions which are relatively common in the Philippines and in the Mediterranean region, respectively **[23]**.

There was also a patient with a typical form of severe Hb H disease with HB 6.7g/dl with low Hb A2, positive Hb H red cell inclusion, and Hb H peak in HPLC but with negative PCR for 21 mutations. This may be due to non-deletion α^+-thalassemia which is relatively uncommon and indicates the need for sequencing of the α-gene locus. There is an inherent difficulty in detecting these mutations because nucleotide sequencing of the α-globin genes with their high GC content is not easy for analysis. In recent years, increasing numbers of non-deletional α^+- thalassemia mutations have been described and more than 30 of these mutations are tabulated in the human globin gene mutation database on the World Wide Web (http://globin. cse.psu.edu).

In our study, we used α-globin Strip Assay for molecular screening of the α-globin gene cluster for the deletional forms of α-thalassemia **[24]** which have some drawbacks as they are able to identify only already defined and common mutations. Other techniques as Multiplex Ligation-dependent Probe Amplification (MLPA) assay **[25-27]**, are useful for detecting unknown and rare α-globin rearrangements (including deletions, triplications and quadruplications) **[28]**.

Chan et al. **[29]** reported that when an individual at reproductive age is found to have Hb H disease, screening his or her partner and other family members for their α-thalassemia carrier status is indicated to carry out DNA-based genotypic analysis regardless of hematologic parameters. If the partner is a carrier of either a deletion or point mutation affecting one single α-globin gene, there is a 25% risk in each pregnancy of conceiving a fetus with Hb H disease. If the partner is homozygous or compounds heterozygous for single α-globin gene

deletion or inactivation, there is a 50% risk that the fetus might have Hb H disease. If the partner is heterozygous for deletion involving both α-globin genes in cis, there is a 25% risk that the fetus will have Hb H disease and another 25% risk that the fetus will have the devastating Hb Bart hydrops fetalis syndrome lacking all α-globin genes **[30]**.

Conclusion:

There is a wide variety of α-thalassemia alleles among Saudi patients, The $-^{3.7}$ is the most common thalassemia mutation in carrier and trait while PA-1 is by far the most common cause of moderate to severe HbH disease phenotype. The α^{0} ($--^{MED}$) allele is also encountered. These data could pave the way for accurate genetic counseling and sound clinical management based on precise molecular diagnosis of α-thalassemia.

Recommendation:

As Hb Bart's hydrops fetalis (homozygosity to an α^{0} defect (--/--) is almost absent in Arabian Peninsula, and Saudi Arabia while HbH (usually due to compound heterozygosity to and α^{0} and α^{+}) is not common **[31]**, so pre marital screening of a couples with unexplained red cells microcytosis and hypochromia, but with normal Hb, iron study and have no H peak in HPLC, and no family history of intrauterine fetal death, chronic anaemia or HGB H disease, they should not be considered for molecular diagnosis, and the counselling should be offered after screening the family with CBC and HPLC, to rule out presence of a patient with HGB H disease.

References

1- AL JAOUNI S.: Prevalence of thalassemia disorders andhemoglobinopathies in Jeddah, western Saudi Arabia. Journal of Applied Hematology, 1: 43-46, 2010.

2- ODED GILAD, ORNA STEINBERG-SHEMER, ORLY DGANY, TANYA KRASNOV C SHARON NOY-LOTAN, HANNAH TAMARY, JOANNE YACOBOVI-CH: Alpha-Thalassemia Carrier due to -α3.7 Deletion: Not So Silent. Acta. Haematol., 143 (5): 432-437, 2020.

3- FUCHAROEN S. and VIPRAKASIT V.: Hb H disease: Clinical course and disease modifiers. Hematology American Society of Hematology Education Program, 26-34, 2009.

4- HIGGS D.R., VICKERS M.A., WILKIE A.O., PRETO-RIUS I.M., JARMAN A.P. and WEATHERALL D.J.: A review of the molecular genetics of the human alpha globin gene cluster. Blood, 73: 1081-1104, 1989.

5- HANAN A. HAMAMY and NASIR A.S. AL-ALLAWI: Epidemiological profile of common haemoglobinopathies in Arab countries. J. Community Genet, 4: 147-167, 2013.

6- IBRAHIM M.GOSADI: National screening programs in Saudi Arabia: Overview, outcomes, and effectiveness. Journal of Infection and Public Health. Volume 12, Issue 5, September-October, Pages 608-614, 2019.

7- SAMANEH FARASHI & CORNELIS L. HARTEVELD: Molecular basis of α-thalassemia. Blood Cells, Molecules and Diseases, 70: 43-53, 2018.

8- HARTEVELD C.L. and HIGGS D.R.: Alpha-thalassaemia. Orphanet J. Rare Dis., 15: 166, 2020.

9- HARTEVELD C.L., VOSKAMP A., PHYLIPSEN M., AKKERMANS N., DEN DUNNEN J.T., WHITE S.J. and GIORDANO P.C.: Nine unknown rearrangements in 16p13.3 and 11p15.4 causing alpha and beta thalassaemia characterised by high resolution multiplex ligation-dependent probe amplification. J. Med. Genet., 42: 922-931, 2005.

10- RANDOLPH T.R.: Thalassemia. In: Clinical laboratory hematology. 3rd ed. New Jersey: Pearson, p. 251-276, 2015.

11- RODAK B.F. and CARR J.H.: Inclusions in erythrocytes. In: Clinical hematology atlas. 5th ed. St. Louis, Missouri: Elsevier Inc., p. 107-14, 2017.

12- https://viennalab.sci-design.com/onlinecalculator/evaluate.php?assay=4-60_20171116.

13- QUADRI M. and ISLAM S.: Hemoglobin H disease in the Eastern region of Saudi Arabia. Saudi Medical Journal, Vol. 21 (7): 666-671, 2000.

14- ARWA Z. AL-RIYAMIA, SHAHINA DAARB, SALAM AL KINDIB, ALI AL MADHANIC, YASSER WALID, MOHAMMED AL RAWAHIA and SHOAIB AL ZAD-JALIA: α-Globin Genotypes Associated with Hb H Disease: A Report from Oman and a Review of the Literature from the Eastern Mediterranean Region. Hemoglobin, Jan. 44 (1): 20-26, 2020.

15- HELLANI A., FADEL E., EL-SADADI S., EL-SWEILAM H., EL-DAWOOD A. and ABU-AMERO K.K.: Molecular spectrum of alpha-thalassemia mutations in microcytic hypochromic anemia patients from Saudi Arabia. Genet Test Mol. Biomarkers., 13: 219-221, 2009.

16- AL-AWAMY B.H.: Thalassemia syndromes in Saudi Arabia. Meta-analysis of local studies. Saudi Med. J., 21: 8-17, 2000.

17- BORGIO J., ABDUL AZEEZ S., ALMANDIL N., NASE-RULLAH Z., AL-JARRASH S., AL-SULIMAN A., EL-FAKHARAY H., et al.: The -α3.7 deletion in α-globin genes increases the concentration of fetal hemoglobin and hemoglobin A2 in a Saudi Arabian population. Molecular medicine reports, 17: 1879-1884, 2018.

18- HAIDER M. and ADEKILE A.: Alpha-2-globin gene polyadenylation (AATAAA->AATAAG) mutation in hemoglobin H disease among Kuwaitis. Med. Princ Pract., 14 (Suppl 1): 73-76. doi: 10.1159/000086187, 2005.

19- MADEEHA KAMAL, RAMIN BADII, ALIAA AMER, SHAZA ABU SIRRIYA and MARINA KLEANTHOUS: The Molecular Basis of Alpha-Thalassemia in the Qatari Pediatric Population. Qatar Foundation Annual Research Conference Proceedings, HBPP1411, 2016.

20- HIGGS D.R., VICKERS M.A., WILKIE A.O.M., PRE-TORIUS I.M., JARMAN A.P. and WEATHERAL D.J.: A Review of the Molecular Genetics of the Human α-

globulin gene cluster. The Journal of The American Society of Hematol., 73: 1081-1108, 1989.

21- HASSAN N.J., KRISHNAMOORTHY R. and AL-ARRAYED S. S.: Molecular basis of α-thalassemia & Hb H disease in Bahrain. First Annual Hematology and Blood Transfusion Meeting, 27 Feb-2 Mar 1999. Cultural Palace, Diplomatic Quarter, Riyadh, Saudi Arabia. Program & Abstracts, p. 91, 1999.

22- LIEBHABER S.A., CASH F.E. and BALLAS S.K.: Human -globin gene expression. The dominant role of the 2-locus in mRNA and protein synthesis. J. Biol. Chem., 261: 15327-15333, 1986.

23- DAVID H.K. CHUI, SUTHAT FUCHAROEN and VIVIAN CHAN: Hemoglobin H disease: Not necessarily a benign disorder. BLOOD, 101 (3): 791-800, 2003.

24- PUEHRINGER H., NAJMABADI H., LAW H.Y., KRUGLUGER W., VIPRAKASIT V., PISSARD S., et al.: Validation of a reverse-hybridization Strip Assay for the simultaneous analysis of common alpha-thalassemia point mutations and deletions, Clin. Chem. Lab. Med., 45 (5): 605-610, 2007.

25- HARTEVELD C.L., VOSKAMP A., PHYLIPSEN M., AKKERMANS N., DEN DUNNEN J.T., WHITE S.J. and GIORDANO P.C.: Nine unknown rearrangements in 16p13.3 and 11p15.4 causing alpha- and beta-thalassaemia characterised by high resolution multiplex ligation-dependent probe amplification, J. Med. Genet., 42 (12) (Dec) 922-931, 2005.

26- LIU J.Z., HAN H., SCHOUTEN J.P., WANG L.R., FAN X.P., DUARTE H.B., et al.: Detection of alpha-thalassemia in China by using multiplex ligation-dependent probe amplification, Hemoglobin, 32 (6): 561-571, 2008.

27- FOGLIETTA E., DEIDDA G., GRAZIANI B., MODIANO G. and BIANCO I.: Detection of alpha-globin gene disorders by a simple PCR methodology, Haematologica, 81 (5) (Sep-Oct) 387-396, 1996.

28- LIU Y.T., OLD J.M., MILES K., FISHER C.A., WEATHERALL D.J. and CLEGG J.B.: Rapid detection of alpha-thalassaemia deletions and alpha-globin gene triplication by multiplex polymerase chain reactions, Br. J. Haematol., 108 (2) (Feb) 295-299, 2000.

29- MIRABILE E., SAMPERI P., DI CATALDO A., POLI A., LA SPINA M. and SCHILIRB G.: Phenotype-genotype correlation in Sicilian patients with Hb H. Eur. J. Haematol., 65: 306-309, 2000.

30- CHAN L.C., MA E.S.K., CHAN A.Y.Y., et al.: Should we screen for globin gene mutations in blood samples with mean corpuscular volume (MCV) greater than 80 fL in areas with high prevalence of thalassaemia? J. Clin. Pathol., 54: 317-320, 2001.

31- HAMAMY H.A. and AL-ALLAWI N.A.: Epidemiological profile of common haemoglobin opathies in Arab countries. J. Community Genet, 4: 147-167, 2013.

توصيف طفرات ألفا ثلاسيميا فى المنطقة الغربية من المملكة العربية السعودية والتوصية بفحص ما قبل الزواج

خلفية الدراسة : تكثر سمة ألفا الثلاسيميا فى المملكة العربية السعودية مع تنوع كبير وتنوع جغرافى.

الهدف من الدراسة : وصف الطيف الجزيئى لمرض الثلاسيميا ألفا فى المنطقة الغربية من المملكة العربية السعودية لدى بعض المرضى السعوديين المشتبه فى إصابتهم بحامل ثلاسيميا ألفا أو المشخصين بمرض هيموجلبين اتش لتحديد ما إذا كان هناك أهمية للاختبار قبل الزواج لطفرات الثلاسيميا بواسطة تفاعل البوليميراز المتسلسل فى الزوجين.

المرضى وطرق الدراسة : تضمنت هذه الدراسة ٣٩ مريضاً، ٣٤ مريضاً يشتبه فى إصابتهم بحامل للثلاسيميا من خلال وجود كريات الدم الحمراء غير المبررة صغيرة الحجم، مع حالة الحديد الطبيعية و5، Hb H أقل من 3.5%، أو كونهم الوالدين المتاحين لمريض مصاب بمرض Hb A2 - α تم فحص ٣٩ مريضاً بحثاً عن ٢١ طفرة HPLC- بواسطة HGB H مرض مصابين بمرض بناء على تفاعل البلمرة المتسلسل globin.

النتائج : هناك ١٣ مريض ٣٣٪ النمط الجينى غير متماثل فى نقص ٣.٧ / و ١٤مريضاً ٣٥.٩٪ متماثل فى نقص ٣.٧.

الاستنتاج : نقص جين ٣.٧ هو الطفرة الأكثر شيوعاً بين مرضى الثلاسيميا ألفا بنسبة ٧٨.٩٪ من جميع عمليات الحذف. لا ينصح بالتشخيص الوراثى الجينى قبل الزواج لمرض الثلاسيميا ألفا فى المنطقة الغربية من السكان السعوديين حيث أن أكثر طفرات ألفا ثلاسيميا شيوعاً هى حذف نقص ٣.٧– والتى لا تشكل خطراً على استسقاء الجنين. يجب النظر فى فحص ما قبل الزواج فى العائلات التى لديها تاريخ مرضى من ألفا ثلاسيميا.