HbH Disease in Bahrain: A Genotype-Phenotype Correlation Report

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ABSTRACT

Objective: To uncover the molecular basis of hemoglobin H (HbH) disease in the population of Bahrain and correlate the underlying genotypes with phenotype presentations.

Design: A retrospective study covering more than 20 years of data collection and analysis for patients having alpha-thalassemia or relevant hematological phenotype manifestations.

Setting: Genetic Laboratory at Salmaniya Medical Complex.

Method: Molecular analysis was established by strip assay analysis targeting specific number of mutations which include the most common α−thalassemia mutations in Bahrain. Confirmation analysis was done by GAP-PCR analysis for the most common deletions ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) and PCR-RFLP analysis for the most common point mutations ($\alpha^{TSaudi}\alpha$ and $\alpha^{Hph}\alpha$). Direct DNA sequencing was accomplished as a final confirmatory step for selected cases. Hematological phenotype analysis was accomplished by using an automated hematology analyzer whereas hemoglobin electrophoresis was accomplished with high performance liquid chromatography (HPLC) system.

Result: Our findings indicate that HbH disease in Bahrain can be broadly categorized into three levels according to the clinical and hematological phenotypes alongside with the underlying genotypes. The first and most severe type of HbH disease is caused by the homozygosity of the Saudi type polyadenylation (polyA) signal mutation (i.e., $\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$); HBA2:c.*94A>G) showing an average level of Hb at 8.5±0.7 g/dL and severe hypochromic and microcytic RBCs with MCH and MCV levels of 18.1±0.5 pg and 60±3.5 fL, respectively. Some of these patients have infrequent blood transfusion and HbH inclusion bodies consistently found on the RBCs in peripheral blood smears after incubation with brilliant cresyl blue stain. The second type of HbH disease is attributed to the compound heterozygosity of the TSaudi haplotype and the pentanucleotide deletion (HBA2:c.95+2_95+6delTGAGG) in α2-globin gene (i.e., the genotype of ($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)) with mean Hb level of 10±0.8 g/dL, and severe level of hypochromia and microcytic anemia at MCH and MCV levels of 18.3±0.9 fL and 58.7±2.6 pg, respectively. These patients rarely need blood transfusion and HbH inclusion bodies occasionally found in RBC peripheral blood smears. The third type, and mildest form of HbH disease in Bahrain, is caused by four different genotypes: ($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$), ($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$), ($-\alpha 3.7/\alpha^{Hph}\alpha$), and ($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$),. These genotypes presented with an average of Hb levels at 10.8±1.0, 10.8±1.2, 10.5±1.6 and 11±1.3 g/dL, respectively. Rarely HbH inclusion bodies can be found in RBCs smears from these patients, and never need blood transfusion due to alpha genotype-related anemia.

Conclusion: This report summarizes the overall phenotype presentations of HbH disease in the population of Bahrain and their various underlying genotypes. This would help in better understanding of the genotype-phenotype correlations in these disorders and improve management and counselling for patients through a better understanding of the disease and relevant pathophysiology.

Keywords: Hemoglobin H, Alpha-Thalassemia, Salmaniya, Hypochromia

INTRODUCTION

Hemoglobin H disease (HbH disease) is a form of phenotypic presentation of alpha-thalassemia and, indeed, it is the most severe viable phenotype of α−thalassemia[1-4]. The naming of this alpha-thalassemia entity (as HbH disease) originate from the beta-globin polypeptidehomotetramer nomenclature [β4; Hemoglobin H (HbH)]. This hemoglobin homotetramer molecule is not existing naturally but due to the severe deficiency in biosynthesis of α−globin polypeptide chain (that are used jointly with the beta-globin chains to form the normal hemoglobin heterotetramer;α2β2) this deficiency in α−chains leads eventually to the accumulation of free and non-linked beta-globin polypeptide chains. Through this dynamic, the accumulated free beta-globin chains will join together in this exceptional circumstances to form the β4-homotetramer molecule (i.e., the Hb H). Nevertheless this abnormal Hb homotetramer do exists in broad spectrum of alpha-thalassemia genotypes with underlying reduction of at least 25% in the alpha-globin polypeptide biosynthesis[5]. This abnormal hemoglobin is physiologically nonfunctional for the intended role of oxygen

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transportation and delivery to various tissues and, indeed, plays a critical role in the premature destruction of affected red blood cells (RBCs) and the pathophysiology in Hb H disease[6,7].

Mendelian traits usually dictated by two genes functioning in trans on two homologous chromosomes, hence the resulting phenotype can be categorized simply into three levels for a typical autosomal recessive trait (like the sickle cell and beta-thalassemia): normal (or wild type homozygous), carrier (or heterozygous) and diseased (or mutant homozygous). In contrast, alpha-thalassemia, and due to the presence of four functioning alpha-globin genes at the same time in the human genome (α1 and α2; in a tandem on each chromosome 16),can be categorized into, at least, 4 levels of α−thalassemia phenotype presentations based on the number of underlying defective (or thereafter normal) alpha-globin genes[3,6]. Nonetheless, further level of complexity do exist due to the differential expression of the two alpha-globin genes described above[8,9].

The first, and the mildest form, of α−thalassemia is the silent α−thalassemia phenotype that is caused essentially by the inactivation or deletion of a single alpha-globin gene out of four[3,6]. This genotype has slight effect, if any, on red blood cell indices (MCV and MCH) without any clinical circumstances. The second level of phenotype presentation is alpha-thalassemia trait phenotype that is caused by inactivation of two (or equivalent of two) alpha globin genes out of 4 with a moderate effect on red blood cell indices and mild-to-moderate level of anemic presentation. The third level of phenotype presentation is HbH disease resulting typically from the inactivation or deletion of three, or equivalent of three, alpha-globin genes out of four. This third level of α−thalassemia presented usually with severe hypochromic and microcytic red blood cells along with moderate-to-severe anemia. Finally, the most severe α−thalassemia phenotype presentation is the α−thal hydrops fetalis syndrome resulting from the deletion of all four alpha-globin genes. This genotype is nonviable and dies in utero in the last stages of pregnancy[10].

Alpha-thalassemia is quite prevalent in the population of Bahrain reaching a remarkable rate of 24%[11,12]. In addition, Hb H disease being long time recognized in Bahrain with sound and clinically varied manifestations[13], however, Hb H disease has not been genotypically categorized in our population. To this end we are presenting here the major Hb H disease-causing α−thalassemia genotypes in the population of Bahrain, along with their various hematological and phenotypic presentations. This would help better understanding of the natural history of α−thalassemia and Hb H disease in our population. Finally, these findings would help in accurate diagnosis and classification of alpha thalassemia and to provide appropriate genetic counseling advice for relevant patients.

METHOD

Patients' samples recruited retrospectively for this study during the previous 20 years of operation in the Genetic Laboratory at SMC. All samples being indicated for alpha-gene analysis due to various levels of anemia unresponsive to iron supplementation along with normal levels of HbA2 and/or family members of alpha-thalassemia patients.

DNA extracted by manual or semi-automated protocols and DNA quality being attested with spectrophotometer measurement at 260 and 280 nm wavelengths. Molecular analysis was established by strip assay analysis for specific number of mutations, including the most common α−thalassemia mutations in Bahrain[14]. Confirmation analysis was done by GAP-PCR analysis for the most common alpha-globin gene deletions ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) and PCR-RFLP analysis for the most common point mutations ($\alpha^{TSaudi}\alpha$ and $\alpha^{Hph}\alpha$)[15-18]. Direct DNA sequencing was accomplished as a confirmatory procedure for selected cases for the various genotypes uncovered.

Hematological phenotype analysis was accomplished by using an automated Coulter hematology analyzer (Beckman Coulter Co., CA, USA) or Siemens Advia hematology analyzer (Siemens Healthcare GmbH, Germany). Hemoglobin electrophoresis was accomplished with the Variant HPLC system (Bio-Rad Laboratories, CA, USA). Brilliant cresyl blue staining was used on selected cases to uncover HbH inclusion bodies display on RBCs blood smear.

Classifications of the various types of HbH disease was based on the alpha-thalassemia genotype uncovered along with clinical and hematological presentation. Statistical values for the different hematological indices were presented as absolute values or as mean ± SD as appropriate. Statistical significance was presented as needed through the Student's T-test with p-value <0.05 indicating a statistically significant difference.

RESULTS

According to the data that we have accumulated, HbH disease or HbH-like disease in Bahrain can be categorized broadly into three types or levels according to the phenotypic presentation and the underlying alpha-globin genotype:

The classical form of HbH disease: Patients in this type display the classical clinical and hematological manifestations of HbH disease that have been described in the literature[1,4]. This type is almost exclusively attributed in our population to the homozygosity of the Saudi-type polyA signal mutation, i.e., the genotype of ($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$), that has been described originally from the eastern part of Saudi Arabia[19,20]. Representative sample of these patients are presented in (Table 1). Their hematological phenotype presentation include severe anemic profile with hemoglobin level mostly less than 9.0 g/dL and HbA2 level less than 2% (Table 1). In addition, they are presented with severe microcytic and hypochromic anemia at an average values of 60 ± 3.5 fL [mean ±SD] for MCV and 18.1 ± 0.5 pg for MCH (Table 2). These patients occasionally need blood transfusion and hospital follow-up, especially during infancy period. In addition, they consistently presented with HbH inclusion bodies on the surfaces of their red blood cells by incubation of their RBCs blood smear with brilliant cresyl blue solution. Pedigrees for Bahraini patients with this genotype, and some other α−thalassemia genotypes, are presented in (Figure 1) showing a typical Mendelian autosomal recessive inheritance found in α−thalassemia.

Moderate form of HbH disease: This type of HbH disease is attributed, on the genotype level, to the compound heterozygosity of the Saudi-type polyA signal mutation and the pentanucleotide deletion (i.e., the genotype of ($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$). These two point mutations represent two of the most common α−thalassemia mutations uncovered in Bahrain[14]. Patients having this genotype are presented mostly with moderately severe level of anemia [Tables 2 and 3], with an average level of Hb at 10 ±0.8 g/dL that is significantly higher than the level found in ($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$) genotype ($p=5.8\times10^{-10}$). Red blood cell indices showed an average of 58.7 ± 2.6 fL for MCV and 18.3 ± 0.9 pg for MCH which are, indeed, not significantly different from ($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$) genotype (p-value > 0.05). In contrast, RBC count and HbA2 levels are found at significantly higher levels than their counterparts in ($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$) genotype ($p= 6.6\times10^{-9}$ and 2.6×10^{-12}, respectively). Rarely any of these patients require blood transfusion and their RBCs blood smear showed occasional and less intense display of HbH inclusion bodies.

Table 1: Representative sample of patients having the genotype of ($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$). These patients presented usually on the clinical and hematological level with classical features of HbH disease.

No.	Gender-Age (y)	Alpha-thal Genotype	Hb (g/dL)	RBC (x10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HbA2 (%)
1	F-47	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	8.7	4.7	64.2	18.5	28.9	1.6
2	M-24	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	8.3	4.5	62	18.4	29.7	1.7
3	F-26	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	8	4.6	60.9	17.2	28.2	1.7
4	F-34	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	8.1	4.4	59.7	18.2	30.5	2
5	F-34	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	7.4	3.9	61.7	18.7	30.2	1.5
6	M-69	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	7.4	4.0	64.5	18.4	28.6	1.6
7	M-35	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	9.6	5.1	59.2	18.7	31.5	1.8
8	F-28	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	9.7	5.3	62.6	18.1	28.9	1.4
9	M-13	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	9.3	5.0	56.1	18.3	32.6	2.1
10	M-11	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	7.8	4.4	64	18	28	1.9
11	M-39	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	8.9	4.9	62.1	18.3	29.4	1.5
12	M-47	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	9.2	5.1	51	18	35	1.9
13	M-9	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	7.8	4.6	52	17	32.7	1.9
14	F-31	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	7.8	4.5	60.8	17.3	28.5	2.2
15	F-35	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	8.2	4.6	59.3	17.7	29.8	1.7
16	F-15	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	8.5	4.6	56.5	18.3	32.4	1.6
17	F-13	($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	8.8	4.85	62.4	18.2	29.1	1.1

Table 2: Overall comparison of hematological phenotype presentations attributed to various underlying Hb H disease-causing genotypes in the population of Bahrain. Representative of the prototypical forms of Hb H disease (due to the genotype of (−α/- -)) and some milder forms of α−thalassemia genotypes are also presented. Normal genotype of the intact four alpha-globin genes [i.e., the genotype of (αα/αα)] with correspondent phenotype is allocated for comparison and reference purposes.

Genotype	n	M/F	Hb* (g/dL)	RBC (x10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HbA2 (%)	α−thallasemia Phenotype
($-\alpha^{4.2}/- -^{Med}$)**	2	1/1	9.8	6.0	64.2	16.5	25.7	2.2	Prototypical form of HbH Disease
($-\alpha^{3.7}/- -^{Fil}$)**	1	F	9.9	6.1	62.0	16.0	26.0	1.7	Prototypical form of HbH Disease
($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$)	32	8/24	8.5 (0.7)	4.6 (0.4)	60.0 (3.5)	18.1 (0.5)	30.2 (1.7)	1.7 (0.3)	HbH Disease
($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	25	8/17	10.0 (0.8)	5.4 (0.5)	58.7 (2.6)	18.3 (0.9)	31.2 (0.8)	2.3 (0.2)	Moderate HbH Disease
($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$)	52	24/28	10.8 (1.0)	5.3 (0.6)	64.1 (2.6)	20.2 (0.9)	31.6 (1.0)	2.6 (0.2)	Mild HbH or α−thal trait
($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$)	27	10/17	10.8 (1.2)	5.5 (0.8)	63.8 (5.3)	19.6 (1.5)	30.8 (1.9)	2.5 (0.2)	Mild HbH or α−thal trait
($-\alpha^{3.7}/\alpha^{Hph}\alpha$)	47	18/29	10.5 (1.6)	4.8 (0.9)	67.5 (4.5)	21.6 (2.1)	31.4 (2.1)	2.7 (0.4)	Mild HbH or α−thal trait
($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$)	19	7/12	11.0 (1.3)	5.6 (0.7)	64.9 (5.9)	19.7 (2.0)	30.6 (2.0)	2.6 (0.3)	Mild HbH or α−thal trait
($-\alpha^{3.7}/-\alpha^{3.7}$)	74	33/41	12.0 (1.4)	5.2 (0.6)	71.2 (3.8)	22.8 (1.4)	32.1 (1.1)	2.6 (0.4)	α−thalassemia trait
($-\alpha^{4.2}/-\alpha^{4.2}$)	16	8/8	12.0 (1.6)	5.2 (0.8)	72.3 (2.5)	22.9 (0.8)	31.5 (1.4)	2.8 (0.3)	α−thalassemia trait
($-\alpha^{3.7}/-\alpha^{4.2}$)	34	16/18	11.6 (1.6)	5.2 (0.7)	70.1 (5.8)	22.4 (2.1)	32.0 (1.4)	2.8 (0.4)	α−thalassemia trait
(αα/αα)	52	19/33	12.7 (1.3)	4.5 (0.5)	84.4 (4.4)	28.2 (2.1)	33.4 (1.5)	2.9 (0.3)	Normal

*Values are mean +/- SD.

**($--^{Fil}$) and ($--^{Med}$) are the Fillibino and Mediterranean type of deletions, respectively, that will remove both α−globin genes (α1 and α2) from the same chromosome.

Mild form of HbH disease: this third category is attributed to at least four different genotypes, ($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$), ($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$), ($-\alpha^{3.7}/\alpha^{Hph}\alpha$) and ($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$). Patients harboring either of these genotypes are presented with mild-to-moderate anemia and Hb level ranging on average from 10.8 ± 1.0 g/dL to 11.0 ± 1.3 g/dL (Tables 2, 4 and 5). None of these patients need blood transfusion and rarely would display HbH inclusion bodies on their RBC blood smear. Nonetheless, these patients would most likely be considered in routine follow-up as having α−thalassemia trait phenotype, rather than HbH disease, due to their milder clinical phenotype presentation. Specific elaboration on various type of $\alpha^{TSaudi}\alpha$ genotype combinations in correlation with phenotype being introduced in a previous publication by our group[21].

In addition, hematological indices and anemia profiles for the following α−thalassemia genotypes: ($-\alpha^{3.7}/-\alpha^{3.7}$), ($-\alpha^{4.2}/-\alpha^{4.2}$) and ($-\alpha^{3.7}/-\alpha^{4.2}$) are also presented for comparison purposes versus other Hb H disease-causing genotype combinations (Table 2). Each of these alpha-thalassemia deletion genotype combinations display typical profile of α−thalassemia trait phenotype. Finally, hematological phenotype for normal and healthy individuals having four α−globin gene complements, i.e., the genotype of (αα/αα), is also presented as the reference profile for normal alpha-globin genes in our population (Table 2).

DISCUSSION

This study reported the most severe, and viable, clinical and hematological phenotype of α−thalassemia in the population of Bahrain, i.e., Hb H disease, correlated with their underlying molecular genotypes. Obviously the most severe α−thalassemia phenotype ever described is the nonviable α−thalassemia hydrops fetalis syndrome that will die in utero or shortly after birth[3,4]. This later syndrome is attributed, on the molecular level, to a complete abolition of the four α−

Table 3: Representative sample of patients having the genotype of ($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$). These patients presented with moderately severe form of HbH disease in comparison with the more severe cases ($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$) genotype.

No.	Gender-Age (y)	Alpha-thal Genotype	Hb (g/dL)	RBC (x10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HbA2 (%)
1	M-45	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	10.9	6.1	57.7	17.8	30.8	2.3
2	F-35	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	9.9	5.5	58.2	18.0	30.9	2.5
3	F-30	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	9.2	4.8	59.4	18.8	31.6	2.4
4	F-18	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	9.6	5.4	57.1	17.6	30.7	2.2
5	F-49	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	10.3	5.3	58.0	19.2	33.1	2.1
6	F-37	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	10.6	6.1	54.9	17.3	31.5	2.4
7	F-10	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	9.2	5.0	58.3	18.3	31.4	2.5
8	F-14	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	9.9	5.4	60.0	19.0	31.0	2.1
9	M-9	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	9.9	5.5	61.0	18.0	30.0	2.2
10	M-9	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	9.0	5.4	55.7	16.8	30.1	2.1
11	M-7	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	9.0	4.9	57.8	18.4	31.9	2.6
12	M-61	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	11.3	6.0	60.0	18.0	30.0	2.3
13	M-40	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	11.9	5.9	63.1	20.1	31.9	2.5
14	F-31	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	8.6	4.6	58.1	18.8	32.3	2.4
15	F-30	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	9.8	5	63.4	19.5	30.8	2.4
16	M-65	($\alpha^{TSaudi}\alpha/\alpha^{Hph}\alpha$)	10.8	6.1	59.5	17.8	29.9	2.3

Table 4: Representative sample of patients having the genotype of ($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$) or ($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$). These patients presented usually with a mild form of HbH disease or α−thalassemia trait phenotype.

No.	Gender-Age (y)	Alpha-thal Genotype	Hb (g/dL)	RBC (x10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HbA2 (%)
1	F-47	($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$)	11.3	5.2	68.4	21.5	31.5	2.6
2	M-21	($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$)	10.2	4.7	63.4	21.3	33.5	2.5
3	F-18	($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$)	10.6	5.0	69.1	20.8	30.1	2.3
4	F-37	($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$)	10.3	4.9	64.2	20.8	32.4	2.5
5	F-39	($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$)	10.1	4.8	60.8	20.6	33.9	2.4
6	F-43	($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$)	10.4	4.9	63.9	21.0	32.9	2.1
7	M-54	($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$)	12.5	5.9	64.3	21.1	32.8	2.6
8	M-54	($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$)	11.3	5.6	61.6	20.0	32.4	2.4
9	F-38	($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$)	11.4	5.9	61.1	19.2	31.4	2.3
10	F-49	($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$)	9	4.2	64.3	21	32.6	2.4
11	F-42	($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$)	10.4	5.3	60	19.3	32.2	2.4
12	M-38	($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$)	12.7	6.7	57.6	18.8	32.6	2.2
13	F-40	($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$)	9.7	5.1	66.1	18.8	28.4	2.6
14	F-48	($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$)	9.6	5.04	57.4	18.9	33	2.7
15	M-54	($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$)	12.7	5.82	66.3	21.7	32.8	2.4
16	M-55	($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$)	11.6	6	64.1	19.1	29.8	2.6

Table 5: Representative sample of patients having the genotype of ($-\alpha^{3.7}/\alpha^{Hph}\alpha$) or ($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$). These patients, as in the case of ($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$) and ($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$) genotypes, are presented with a mild form of HbH disease or α−thalassemia trait phenotype.

No	Gender-Age (y)	Alpha-thal Genotype	Hb (g/dL)	RBC (x10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HbA2 (%)
1	M-24	($-\alpha^{3.7}/\alpha^{Hph}\alpha$)	11.9	5.3	71.7	22.2	31	2.8
2	F-34	($-\alpha^{3.7}/\alpha^{Hph}\alpha$)	10	4.4	71.8	22.4	31.2	2.6
3	M-34	($-\alpha^{3.7}/\alpha^{Hph}\alpha$)	13	5.9	68	21.8	32	2.3
4	M-48	($-\alpha^{3.7}/\alpha^{Hph}\alpha$)	12.1	5.8	68.7	20.7	30.2	2.3
5	F-21	($-\alpha^{3.7}/\alpha^{Hph}\alpha$)	10.4	4.92	66.1	21.1	31.9	2.3
6	F-26	($-\alpha^{3.7}/\alpha^{Hph}\alpha$)	12.5	6.24	65.5	20	30.5	2.7
7	F-44	($-\alpha^{3.7}/\alpha^{Hph}\alpha$)	10.2	4.57	77	22	29	2.5
8	F-25	($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$)	10.7	5.4	65.5	19.8	30.3	2.8
9	M-26	($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$)	12.4	6.34	63.6	19.5	30.6	2.8
10	M-10	($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$)	10.6	4.78	67.7	22.2	32.7	2.7
11	F-31	($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$)	10.7	4.91	67.1	21.7	32.4	3
12	F-41	($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$)	10.4	5.9	67.4	17.5	25.9	2.4
13	M-7	($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$)	10.5	5.5	63.1	19.2	30.4	2.2
14	F-6	($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$)	10.3	6.1	60.4	16.9	28	2.9

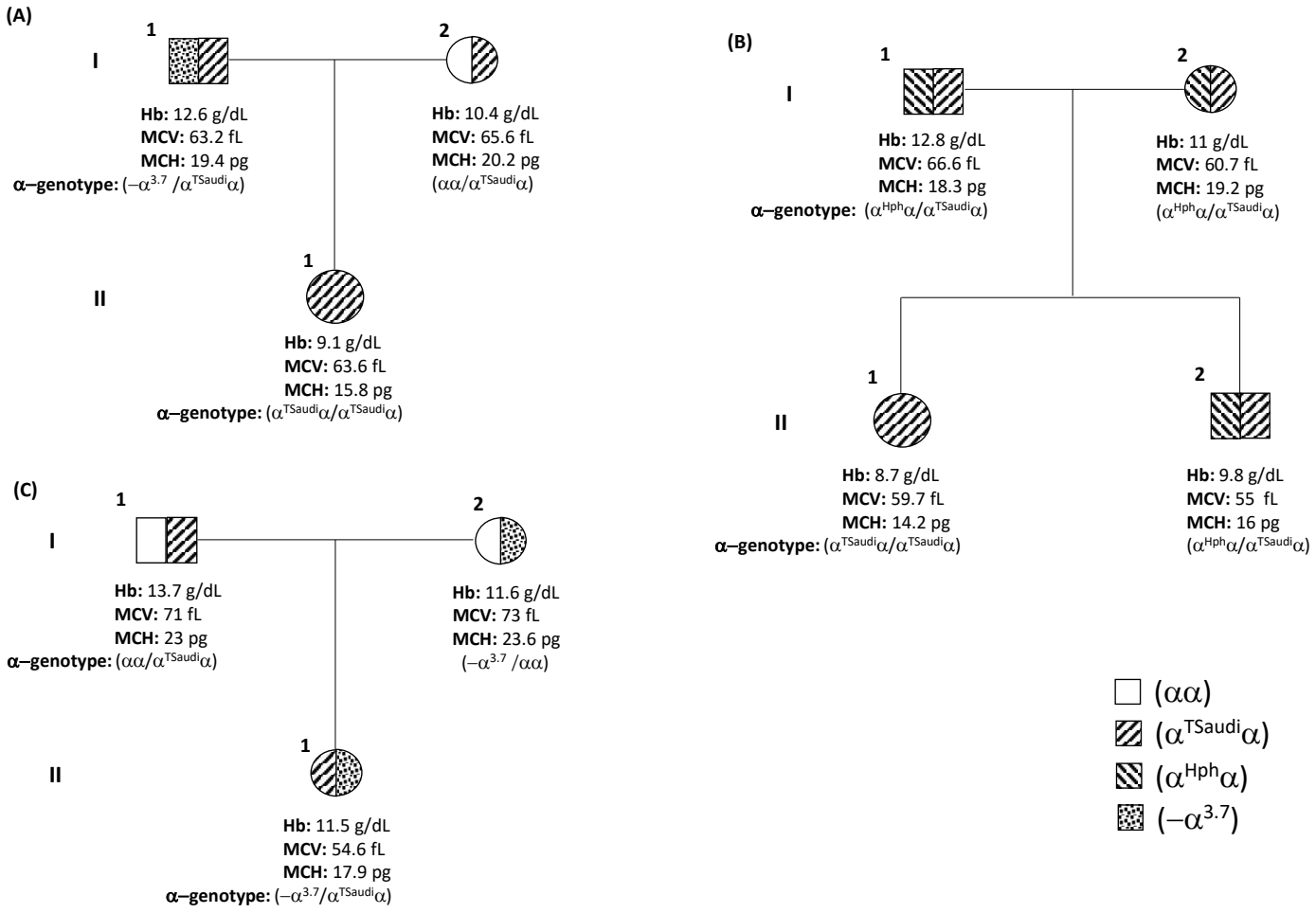

Figure 1: Representative α−thalassemia pedigrees with various underlying α−genotypes including some genotype combinations leading to HbH disease. Alpha thalassemia follow a typical inheritance profile for a Mendelian autosomal recessive (AR) trait. For example in family A the α−thalassemia genotype probability in each pregnancy is distributed as follows: 25% $-\alpha^{3.7}/\alpha\alpha$, 25% $\alpha^{TSaudi}\alpha/\alpha\alpha$, 25% $\alpha^{TSaudi}\alpha/\alpha^{Tsaudi}\alpha$ and 25% $-\alpha^{3.7}/\alpha^{TSaudi}\alpha$. Generations are indicated with Greek numerals and family members per generation with Arabic numerals. Alpha genotypes and anemia levels are noted down for each family member.

globin genes on chromosomes 16[1]. However, this underlying genotype of α−thalassemia hydrops fetalis syndrome would mostly result from the existence of type-1 α−thal mutations, i.e., the intact deletion of both α−globin genes, α1 and α2, on the same chromosome, in the population. Up to our knowledge, type-1 deletion of α−thalassemia mutation does not exist[14], or extremely rare, in the population Bahrain versus some other parts of the world like South East Asia[3,4]. This conclusion regarding existence of the type-1 α−thal deletion in Bahrain, versus type-2 deletion, which is mostly represented by $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions, can be confidently reached through our long time observation and analysis of α−thalassemia on the molecular level in Bahrain. For more than 20 years of investigating thousands of α−thal cases in our population we hardly encountered type-1 deletion; and the few cases uncovered so far are mostly for expatriate working in Bahrain, and in the majority of cases for patients coming from South East Asia (Unpublished Data).

Given the high frequency of α−thalassemia in the population of Bahrain[11,12], our finding in this report would be significant in sorting out the molecular basis of the most prominent and clinically sound phenotype of α−thalassemia in our population. This would be important for both definitive diagnosis of HbH disease and for follow up of patients all based on best understanding of the pathophysiology in α−thalassemia. For instance, one of the major issues in follow up of such cases is iron status and the need to prevent further aggravation of the anemic phenotype through concurrent iron deficiency anemia. On the other hand, and due to the inherent genetics of α−thalassemia, one would not be able to entirely correct the anemic status in these patients. Eventually, the follow-up physicians need to be cautious enough not to cause an iron overload due to over prescription of iron supplement for patients who have inherent deficit of α−globin gene expression especially for the more severe α−genotypes to handle extra iron uptake.

Our finding in this report revealed that the most severe presentation of HbH disease in Bahrain is attributed largely to the homozygous genotype of TSaudi polyA signal mutation (i.e., the genotype of $\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$). Similar findings being uncovered in some other regional countries with reported high frequency of the TSaudi polyA signal mutation[22-24]. This exceptionally prominent severity of the polyA signal was, indeed, a surprising finding at first discovery of this mutation in correlation with Hb H disease in 1980s[19,20,25]. In other word, it was well established, as mentioned above, that HbH disease is attributed historically to the abolition of three α−globin genes out of four (i.e., the genotype of (–α/--)) whereas the genotype of ($\alpha^{TSaudi}\alpha$/

$\alpha^{TSaudi}\alpha$) has an apparent inactivation of only two α−globin genes rather than three. In theory, for this genotype of homozygous polyA signal mutation the most expected phenotype would be an α−thalassemia trait presentation rather than HbH disease. It turned out that the most plausible explanation for this phenotype is that this genotype is not solely inactivating a single α2−globin gene per chromosome 16. Rather, there is a negative effect on the linked α−1 globin gene through an α2-mRNA read-through mechanism that will affect normal transcription of the downstream α1-globin gene as well[25,26], and this theory being, indeed, supported experimentally[27,28]. In summary, this theory imply a continuous transcription of the α−2 globin gene, due to the mutated polyA signal, into the downstream α1-globin gene subunit and effectively interfering with normal transcription of that gene. In essence this mutation will eventually lead to a defective transcription of both α2-and α1-globin genes[25,26]. Hence the severe presentation found in the genotype of ($\alpha^{TSaudi}\alpha/\alpha^{TSaudi}\alpha$) is mostly attributed to this pathophysiologic mechanism that will produce level of α-globin gene polypeptide less than what is expected from a single α1-globin gene per chromosome 16.

In the third category of HbH disease in Bahrain, i.e., the mild form of HbH disease, the underlying genotypes attributed to either coinheritance of TSaudi mutation with $-\alpha^{3.7}$ deletion ($-\alpha^{3.7}/\alpha^{TSaudi}\alpha$) or $-\alpha^{4.2}$ deletion ($-\alpha^{4.2}/\alpha^{TSaudi}\alpha$) or the homozygosity of the pentanucleotide deletion (i.e., $\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$). For the first two genotypes (i.e., $-\alpha^{3.7}/\alpha^{TSaudi}\alpha$ and $-\alpha^{4.2}/\alpha^{TSaudi}\alpha$) the increased severity can be attributed principally to the $\alpha^{TSaudi}\alpha$ allele. This can be clearly appreciated by comparing these two genotypes with the $-\alpha^{3.7}/-\alpha^{3.7}$ and$-\alpha^{4.2}/-\alpha^{4.2}$ as well as $-\alpha^{3.7}/-\alpha^{4.2}$ phenotype presentation (Table 2). These three later genotypes are presented with α−thalassemia trait phenotype rather than HbH disease and significantly milder anemic presentation (p-value < 0.05 for Hb levels) (Table 2). For the third genotype in this category, i.e., the ($\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$) genotype, this pentanucleotide deletion mutation would specifically inactivate α2-globin gene which is more important in the context of expression versus α1-globin gene[29]. In contrast, the $-\alpha^{4.2}$ deletion is removing the entire α2-globin gene and sparing α1-globin gene[30], however the severity in $-\alpha^{4.2}/-\alpha^{4.2}$ is, indeed, not reaching that in $\alpha^{Hph}\alpha/\alpha^{Hph}\alpha$ genotype (Table 2). This might be attributed, at least partially, to the compensatory effect on the expression capacity of the linked α1-globin gene due to the large structural changes related to this deletion of 4.2 kb. In contrast the small α2 intragenic deletion of just five nucleotides in $\alpha^{Hph}\alpha$ allele is behaving like a point mutation and presumably inactivating α2-globin gene solely without an appreciated effect on the expression output from the linked α1-globin gene.

LIMITATIONS AND RECOMMENDATIONS

One major limitation of this study is the lack of thorough clinical data for the relevant patients. It would be very useful to find exact clinical manifestations that might be correlated with specific genotype combinations (e.g., gallstones and hepatosplenomegaly). Another point of limitation is to find out age-related clinical manifestations for these genotypes, and in this case we need to follow-up various age group per Hb H genotype to find out such diverted clinical manifestations if any. It would be also very much helpful to find levels of Hb H fraction of hemoglobin hemolysate in these patients as a further evidence of α−polypeptide deficiency for the various genotypes. Finally it would be very interesting to find out effect of these genotypes on the clinical manifestations of sickle cell disease especially with regard to the well reported ameliorating effect of alpha-thalassemia on SCD.

CONCLUSION

Hemoglobin H (Hb H) disease in Bahrain can be categorized broadly into three types: classic, moderate and mild forms according to the underlying genotype and relevant phenotype. This report present important information to fill up the genotype-phenotype correlation gaps in the sake for a better understanding of Hb H disease in Bahrain and for a rational genetic counseling in affected families.

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