Three Clinical Types of Sickle Cell-Beta-Thalassemia Disease in Bahrain: Dictating Role of Beta-Thalassemia Mutations

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ABSTRACT

Our objective in this report is to investigate beta-globin genotypes and the correlated phenotypes in sickle cell-beta-thalassemia (S-beta-thal) patients in Bahrain. A retrospective study and review of the EHR for patients with S-beta-thal uncovered during student screening project and premarital counseling that undergo full molecular analysis.Genetic Laboratory at Salmaniya Medical Complex. Full molecular analysis of the beta-globin gene was accomplished by using ViennaLab beta-thalassemia kit and RFLP analysis for the most common beta-thalassemia mutations in Bahrain. Hematology analyzer and HPLC instrument was used for blood phenotype analysis. Patients' clinical profiles obtained through the Electronic Health Record (I-Seha). We uncovered clinically three distinct forms of S-beta-thal with underlying five different beta-globin genotypes: first, a severe type of S-beta-thal [n=31] with underlying beta-thal mutations mainly of codon (Cd) 39 (HBB:c.118C>T) [n = 18; 58 %], or the IVS I,3'end (-25 bp deletion) (HBB: c.93-22_95del) [n = 13; 42 %], along with the sickle mutation in trans. Second, a moderate form of S-beta-thal [n=12] attributed to the coinheritance of nucleotide (nt) -88 (C>A) (HBB:c.-138C>A) [c.-138C>A] and the sickle cell mutation. Third, a milder form of s-beta-thal [n=24] with either nt -101 (C>T) [HBB: c.-151C>T] [n = 11] or nt -71 (C>T) [HBB: c.-121C>T] [n = 13], and the sickle cell mutation. The first type displays HbSS profile of Hb-electrophoresis with the absence of normal adult hemoglobin (HbA) and presence of various levels of HbS (Range: 57.6 % - 85.1 %) and elevated HbF (3.5% - 36.1%). The second type shows moderate Hb-electrophoresis profile with mean HbS of 61.7 ± 7.6 % and significant amount of HbA at 23 ± 6.1 %. The third type presents with distinct Hb-electrophoresis pattern resembling, in essence, Hb electrophoresis profile of sickle cell trait showing mean HbA of 45 ± 3.1 % and HbS of 41.6 ± 2.5 %. These findings would be invaluable for better understanding of the nature of S-beta-thal disease in Bahrain with implications for clinical follow up and premarital counselling for patients and their families.

Keywords: Bahrain; Sickle cell disease (SCD); sickle cell-beta-thalassemia (s-beta-thal); HbS-beta-thalassemia (HbS-beta-thal); beta-thalassemia.

INTRODUCTION

Sickle cell disease is divided categorically, based on the underlying genotype combination, into two major subtypes: the homozygous SS genotype (sometimes referred to as sickle cell anemia), and the sickle cell-beta-thalassemia (S-beta-thal) disease which is a compound heterozygous condition of sickle cell and the beta-thalassemia mutations in trans[1]. Both of these defined disease genotypes (i.e., SS and S-beta-thal) are called off as sickle cell disease (SCD), and guidelines do exist for the diagnosis and screening for the various type of sickle cell disease including some minor genotypes combinations, such as the HbSC or HbSD genotypes[2,3].

Existence of both sickle cell mutation and the various type of beta-thalassemia mutations in a specific population would obviously pave the ground for generation of such genotype combinations as found in S-beta-thal disease. This versatile genetic background do exists indeed in the population of Bahrain, having both the sickle cell mutation, which is quite prevalent in this population at ~11%[4,5,6], alongside with various type of beta-thalassemia mutations that do occur albeit with a much lower frequencies at 2.1-2.9%[4,5,6]. Consequently this would pave the ground by this population genetic makeup to uncover rare underlying combinations of the sickle cell and beta-thal genotypes, which we aim in this report to characterize and correlate their phenotypic presentation with the underlying genotypes.

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Since the underlying sickle cell mutation is the common gene defect amongst the various type of sickle cell-beta thal disease, the phenotypic variation of S-beta-thal is very much dictated, presumably, by the type of underlying beta-thalassemia mutation. Nonetheless other genetic and environmental factors, including type of sickle cell haplotype, being cited as modifiers in sickle cell disease[2,7]. However, in regard of the sickle cell haplotype in Bahrain the underlying HbS haplotype is, indeed, quite homogenous being of the Arab-Indian (AI) haplotype in the vast majority of cases reported[8], rather than any of the various African HbS haplotypes[7,9]. Again these genetic background makes the variation in phenotypic presentation in S-beta-thal diseases in Bahrain largely dictated by the type of beta-thalassemia mutation rather than the sickle cell haplotype.

In summary, our objective in this report is to uncover and analyze the various type of S-beta-thal disorders in the population of Bahrain, and to correlate their phenotypic presentation with the underlying beta-globin genotypes.

MATERIALS AND METHODS

This study is a retrospective investigation, and patients were recruited mainly through a student screening project, with a targeted 7000 students per year for the 11th grade level as reported before[10], and patients seeking a premarital and genetic counseling services through the Genetic Department at SMC. Samples for students and counselees with suspected and atypical HPLC profiles were sent to the genetic laboratory at SMC for further molecular investigations and confirmation. A total number of 67 patients with various types of SCD being selected for this study as follows: 31 patient with severe form of SCD, 12 with moderate form of SCD, and 24 patients with mild form of SCD. General consents were obtained from all participants in this study and patients' samples collected in accordance with the Declaration of Helsinki.

Complete blood count (CBC), including reticulocyte counting, was accomplished by using a certified automated hematology analyzer (Beckman Coulter Co., CA, USA). Hemoglobin (Hb) electrophoresis profile was obtained by using Bio-Rad's Variant high performance liquid chromatography (HPLC) analyzer (Bio-Rad Laboratories, Hercules, CA) on freshly collected whole blood hemolysate. Biochemical analysis was accomplished by using automated blood chemistry analyzer (Cobas Analyzer, Roche Diagnostics International AG, Switzerland) as described before[11].

Patient-specific clinical data were retrieved from the national electronic health records (I-Seha) for the allocated period of time (Jan 2018 - Jan 2022). Standard criteria was used for the characterization of the different clinical episodes (12). Molecular analysis of the beta-thalassemia and sickle cell mutations was accomplished by using a reverse-dot blot-based commercial kits (Beta-Globin StripAssay; ViennaLab Diagnostics GmbH, Austria) essentially as described before[11,13]. Restriction fragment length polymorphisms (RFLPs) procedure was employed to uncover nucleotide -71 (C-T) mutation (c.-121C>T) as published before[13]. Finally, Sanger sequencing with either the CEQ 8800 instrument (Beckman Coulter Co., USA) or the ABI 3500 instrument (ThermoFisher Co., USA) was used to uncover nucleotide -88 (C-A) mutation (c.-138C>A), and to confirm other uncovered beta-thalassemia mutations in selected patients, as described before[11].

Continuous data variables (e.g., Hb levels, CBC indices, etc…) are presented as mean +/-SD, and statistical significance was calculated by using Student T-test with $p < 0.05$ considered significant. Categorical variables (e.g., VOC frequency, admission frequency, etc) are presented as percentage, and differences significance was calculated by using Fisher exact test. Microsoft's Excel spread sheet and a freely available social statistics website (https://www.socscistatsitics.com) was used for the calculations of the various statistical parameters.

RESULTS

Phenotype of S/25 bp del versus S/Cd39: Both of these genotypes show the typical profile of Hb-electrophoresis found in sickle cell anemia (S/S) with complete absence, as expected, of the major adult hemoglobin (HbA) and the presence of only HbS fraction, along with various levels of fetal hemoglobin (HbF), and minor adult hemoglobin (HbA2) (Tables 1 and 2). However, HbF show a higher trend, although not significant ($p > 0.05$), in S/25 bp del S-beta-thal genotype vs. S/Cd 39 genotype (Table 1 and 2). Other hematological findings and indices revealed an almost similar profiles between these two genotypes with the exception of HbA2 level which showed a significantly higher levels in S/Cd39 vs S/25 bp del ($p<0.05$) (Table1 and 2). In addition, the display of clinical findings indicate a general trend of relatively milder profile for S/25 bp del genotype for most parameters analyzed during the indicated 5 years period versus S/Cd 39 (Table 3). This includes events of vaso-occlusive crises which inflicted 94% (17/18) of patients with the genotype of S/Cd 39 (Table 3). In contrast, patients with the genotype of S/25 bp del have VOC episodes in 69% (9/13) of the cases

Table 1. Hematological parameters (HPLC & CBC) for the compound heterozygous condition of the S/25 bp

Age (M/F)	HbA2 (%)	HbF (%)	HbS (%)	HbA (%)	Hb (g/dL)	RBC (x10^{12}/L)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Retics (%)
20 (M)	4.6	28.4	65.9	-	10.8	5.2	34	66.1	21.2	31.8	3.5
14 (M)	4.2	21.7	73.5	-	10.7	4.6	34.7	75.2	23.1	30.7	2.1
14 (F)	4.2	28.9	65.6	-	9.8	4.7	28.7	60.8	20.8	34.1	1.8
11 (F)	5.5	26.9	65.8	-	11.4	4.8	33.1	69	23.7	34.3	1
38 (F)	5.7	13.8	78.3	-	9.6	3.9	30.6	78.7	24.6	31.2	4.2
36 (F)	4.5	36.1	58.5	-	11	4.2	32.9	77.5	25.8	33.3	3.5
36 (F)	5.6	10.4	83.1	-	9.7	4.5	28.9	63.5	21.3	33.6	5
31 (F)	4.5	11.1	83.4	-	9	4	26.8	67.5	22.7	33.6	3.6
30 (M)	5.7	12.7	80.4	-	10.7	5	35	69.8	21.3	30.6	4
28 (F)	7	15	74	-	9.9	4.3	29.9	70.4	23.3	33.1	4
37(F)	5.2	12.4	77.9	-	8.8	3.74	25.5	68.1	23.4	34.4	1.8
15(M)	5.4	16.2	76	-	9.9	4.8	29	60.2	20.5	34.1	5.6
23(M)	4.8	18.3	74.9	-	9.7	4.6	27.9	60.5	21	34.8	2.8
Mean*	**5.1±0.8**	**19.4±8.3**	**73.6±7.6**	**-**	**10.1±0.8**	**4.5±0.4**	**30.5±3.1**	**68.3±6.2**	**22.5±1.6**	**33.0±1.5**	**3.3±1.3**

del S-beta-thal genotype, *Mean ± SD.

(Table 3). Moreover, patients with the S/25 bp del have fewer frequency of hospital admission, which turned out to be significant (P<0.05), and less frequently in needs of blood transfusion versus patients with the genotype of S/Cd 39 (Table 3). Finally, the number of patients with prescription for hydroxyurea was found to be higher, although statistically not significant, for the genotype of S/Cd 39 vs the genotype of S/25 bp del (Table 3). Overall, these clinical phenotypic differences might be attributed, at least partially, to the varied HbF expression that is observed between these two genotypes (Table 1 and 2).

Phenotype of S/-88 versus both S/-101 and S/-71: Sickle cell group with the genotype of S/-88 has a distinct Hb-electrophoresis profile showing significant output of adult major hemoglobin (HbA) at 23 ± 6.1% of total hemoglobin (Table 4). This magnitude of HbA level stands, indeed, in the middle amongst other types of SCD; with total absence of HbA in the severe type of SCD (Table 1 and 2), and a much higher level for both S/-101 and S/-71 at 45 ± 3.1% on average (Table 5,6). In addition, S-beta-thal with the genotype of S/-88 show higher levels, although statistically not significant, of HbF expression at 7.4 ± 3.8% versus 4.4 ± 2.9 % on average for both S/-101 and S/-71 [p > 0.05] (Tables 4,5 and 6)[12]. These findings reflect the relatively moderate effect of nt -88 C>A mutation on beta-globin gene expression versus the milder effect of nt -71C>T and -101 C>T on beta-globin gene output expression. In addition, the S/-88 genotype underlies a modest increase in clinical severity versus the genotypes of S/-101 and S/-71. This includes, for instance, only one case having VOC for S/-88 versus no VOC episodes encountered in patients with the genotypes of S/-101 and S/-71 for the entire 5-years period under investigation (Table 3). Moreover, only one case with the genotype of S/-88 (8%) required hospital admission and blood transfusion during that period of time which was, indeed, related to pregnancy complications (Table 3). In contrast, SCD with the genotypes of S/-101 and S/-71 presented clinically with essentially asymptomatic profile of sickle cell disease which, in essence, resemble the phenotype of sickle cell trait (SCT) albeit with distinct Hb electrophoresis profile (Table 5,6) (see Hb electrophoresis profiles for SCT in Ref 13 for comparison). A comparable and overall Hb-electrophoresis profiles of the various S-beta-thal genotypes described in this report are presented in Figure 1.

DISCUSSION

Sickle cell disease is a major burden on health care services in the Kingdom of Bahrain. For instance, in the year 2019 (directly before the COVID-19 pandemic) the second most admitted patients in the major public hospital in this country (Salmaniya Medical Complex) are attributed to SCD and its complications at 9.5% of total discharge[14]. Although S-beta-thal, as one type of SCD, has been described previously on the genotype and phenotype level for different populations[15,16,17], deciphering the various types of S-beta-thal disorder in the population of Bahrain on the level of genotype and phenotype has not been described. In this study we report the various type of sickle cell-beta-

Table 2. Hematological parameters (HPLC & CBC) for the compound heterozygous condition of the S/Cd 39 (C>T) S-beta-thal genotype

Age (M/F)	HbA2 (%)	HbF (%)	HbS (%)	HbA (%)	Hb (g/dL)	RBC (x10^{12}/L)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Retics (%)
19 (F)	5.9	17.2	75.6	-	9.8	4.4	33	78.1	23.1	29.5	4.2
20 (M)	6.5	12.1	76.4	-	9.9	4.3	32	74.6	23.2	31.1	6.3
49 (F)	5.2	11.4	80.8	-	7.9	3.7	24.8	67.7	21.6	31.9	5.6
54 (F)	5.1	23.4	70.4	-	9.4	3.9	28.5	73.8	24.4	33	2
20 (M)	6.2	17.7	74.5	-	10.8	5.1	33.7	66.3	21.3	32.1	2.7
10 (F)	5.8	19.4	73.2	-	9.2	4.2	28.9	68.2	21.7	31.9	2.9
38 (F)	7.3	10	77.8	-	9.4	4.1	30.8	74.6	22.8	30.6	4
36 (F)	4.7	19.8	74.5	-	7.5	3.4	23.1	67.6	22	32.5	5.3
36 (F)	6.4	16.1	76.2	-	9.4	4.1	29.6	64.6	22.8	35.3	3.2
37 (M)	6.8	14.1	77.2	-	12.2	4.2	34.2	80.9	28.8	35.7	1.8
46 (M)	6.8	5.2	85.1	-	10.3	4.5	32.9	72.3	22.7	31.4	4.4
52 (M)	5	20.5	71.8	-	9.1	4	28	69.9	22.7	32.5	5.6
43 (F)	5.2	9.5	82.3	-	8.7	3.9	27.2	69.1	22.2	32.1	6.6
53 (M)	5	29.9	62	-	13.4	6	40.5	67.9	22.5	33.1	3.9
46 (M)	6.5	13.9	77.5	-	11.2	4.6	33.7	73	24.3	33.3	3
43 (F)	5.4	31.9	62.3	-	9	3.6	29.6	83.4	25.2	30.2	5.2
44 (F)	8	3.5	84.5	-	9.4	5.1	31.9	62.7	18.4	29.4	1.9
Mean*	**6.0±0.9**	**16.2±7.7**	**75.4±6.4**	**-**	**9.8±1.5**	**4.3±0.6**	**30.7±4**	**71.5±5.7**	**22.9±2.1**	**32.1±1.7**	**3.8±1.8**

*Mean ± SD; NA, not available.

Table 3. Number of patients having either of the various clinical episodes enlisted, irrespective of frequency, for the indicated S-beta-thal genotypes.

	Genotype	n	VOC1[1]	%	Admission[2]	%	Tx[3]	%	HU[4]	%
1	S/25 bp del	13	9/13	69	4/13	31	May-13	38	2/13	15
2	S/Cd 39	18	17/18	94	14/18*	78	10/18	56	7/18	39
3	S/nt-88	12	2/12	17	1/12	8	1/12	8	0/12	0
4	S/nt-71	13	0/13	0	0/13	0	0/13	0	0/13	0
5	S/nt-101	11	0/11	0	0/11	0	0/11	0	0/11	0

[1]VOC, number of patients documented with vaso-occlusive crises from Jan 2018 to Jan 2022; [2]number of patients with the need for hospital admission during the 5 years period from Jan 2018-Jan 2022; [3]Tx, number of patients transfused in the period of 5 yrs (2018-2022); [4]HU, hydroxyurea prescription; *P < 0.05 for S/Cd 39 vs. S/25 bp del.

Table 4. Hematological parameters (HPLC & CBC) for the compound heterozygous condition of S/nt-88 S-beta-thal genotype.

Age	HbA2	HbF	HbS	HbA	Hb	RBC	Hct	MCV	MCH	MCHC	Retics
(M/F)	(%)	(%)	(%)	(%)	(g/dL)	(x10^{12}/L)	(%)	(fL)	(pg)	(g/dL)	(%)
26 (F)	6.5	10.5	58.2	23.2	10.9	4.9	31.3	63.9	22.2	34.8	1.1
21 (M)	3.9	10.8	39.9	41.8	13.7	5.7	41.8	73.2	24	32.8	1.8
48 (F)	5.9	7.2	66.1	20.6	9.4	na**	28	81.9	27	33.1	na
12 (M)	5.2	16.1	60.4	16	12.4	5.1	38.6	76	24.5	32.2	2.1
26 (F)	5.3	5.5	66.3	20.4	10.4	4.6	35	76.4	22.7	29.7	2.2
24 (M)	7.4	5.5	65.7	19.6	12.3	5.9	36	60.7	20.7	34.2	na
41 (F)	5	1.3	70.9	22.1	9.4	5	30.6	61.7	18.9	30.7	3.2
36 (F)	8	4.4	60	24.8	12.5	5.7	36.6	64.6	22.1	34.2	na
37 (M)	6.5	3.2	66.9	22.3	15.5	6.2	48	76.3	24.9	32.6	na
14 (F)	6.4	8	66.3	19.1	10.6	4.1	31.6	76.3	25.5	31.1	1.8
40 (F)	7	8.9	60.2	23.3	13.6	5.8	40	69.2	23.5	33.9	2.7
42 (F)	7.7	7.1	58.9	23.3	11.2	5.2	36	68	21	31	1.2
Mean*	**6.2±1.2**	**7.4±3.8**	**61.7±7.6**	**23.0±6.1**	**11.8±1.8**	**5.3±0.6**	**36.1±5.3**	**70.7±6.6**	**23.1±2.2**	**32.5±1.6**	**2.0±0.7**

*Mean ± SD; ** na, Not available.

Table 5. Hematological parameters (HPLC & CBC) for the compound heterozygous condition of S/nt-101 S-beta-thal genotype.

Age	HbA2	HbF	HbS	HbA	Hb	RBC	Hct	MCV	MCH	MCHC	Retics
(M/F)	(%)	(%)	(%)	(%)	(g/dL)	(x10^{12}/L)	(%)	(fL)	(pg)	(g/dL)	(%)
31 (F)	4.2	5.5	41.9	43.9	12.3	5.4	39.3	73	22.9	31.3	na
13 (F)	na**	na	na	na	11.8	5.8	37.8	65.1	20.3	31.1	na
11 (M)	4.3	5.1	41.4	45.6	12.4	5.1	36.4	71.4	24.3	34.1	na
17 (F)	4.1	2.4	34.8	52.8	11.5	5.7	36	63.6	20.3	31.9	na
13 (F)	na	na	na	na	11.8	5.8	37.8	65.1	20.3	31.1	na
62 (M)	3.6	3.8	40	47.2	12.9	5.4	38.3	70.7	23.8	33.7	na
4 (M)	3.5	10.6	41.8	41.2	12.1	4.9	35	71.8	24.7	34.4	0.8
39 (M)	3.7	3.8	41.2	43.5	16.5	6.3	49	78	26	33	na
37 (F)	4.2	2.2	42.5	46.8	13.5	5.1	42	82	26	33	na
37 (M)	na	3.7	44.5	43.3	15.1	5.3	44	82.6	28.5	34.6	na
62 (M)	3.6	3.8	40	47.2	12.9	5.4	38.3	70.7	23.8	33.7	na
Mean*	**3.9±0.3**	**4.5±2.5**	**40.9±2.7**	**45.7±3.4**	**13.0±1.5**	**5.5±0.4**	**39.4±4.1**	**72.2±6.5**	**23.7±2.7**	**32.9±1.3**	**0.8**

*Mean ± SD; ** na,Not available.

Table 6. Hematological parameters (HPLC & CBC) for the compound heterozygous condition of the S/nt-71 S-beta-thal genotype.

Age	HbA2	HbF	HbS	HbA	Hb	RBC	Hct	MCV	MCH	MCHC	Retics
(M/F)	(%)	(%)	(%)	(%)	(g/dL)	(x10^{12}/L)	(%)	(fL)	(pg)	(g/dL)	(%)
44 (M)	4.1	5.2	45.1	41.8	14.7	5.1	43.1	84.5	28.8	34.1	na**
39 (M)	4.4	1	44.4	45.3	13.9	6	41.7	70	23.3	33.3	na
39 (F)	4.8	3.8	43.7	43.6	12.3	4.5	36.7	82.3	27.6	33.5	na
39 (F)	4.2	3.4	36.5	49.6	11.3	4.6	32.1	68.9	24.3	35.3	1.6
39 (F)	na	2.8	39.2	48.3	12.2	5.5	38.1	69.7	22.2	31.9	na
36 (F)	3.3	9.5	40.1	40.9	13.4	4.9	41	83.5	27.4	32.9	na
36 (F)	3.5	10.3	42.5	40.5	12	4.6	35.9	77.2	25.8	33.4	na
37 (F)	5.1	1.1	40.5	50	12.8	4.9	39.3	80.8	26.3	32.6	na
36 (M)	4.5	3.4	45.4	42.2	15.8	5.8	48	83	27	33	na
37 (F)	4.7	2.6	38.7	49.6	12.4	4.6	38	83	27	32	na
36 (F)	3.7	4.6	42	43.8	12	4.9	37	75.7	24.6	32.5	na
36 (F)	3.7	2.9	38.8	46.6	12.8	5.1	38	75	25	33	na
Mean*	**4.2±0.6**	**4.2±2.9**	**41.4±2.9**	**45.2±3.6**	**13.0±1.3**	**5.0±0.5**	**39.1±4.0**	**77.8±5.9**	**25.8±1.9**	**33.1±0.9**	**1.6**

*Mean ± SD; **na, Not available.

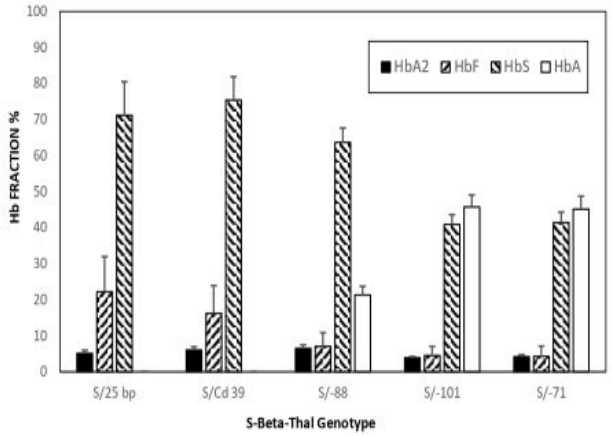

Figure 1. Hemoglobin electrophoresis profiles for the various S-beta-thal genotypes described in this article

thal disease in Bahrain which contribute to the overall burden of SCD in this country. However, given the low frequency of beta-thalassemia trait in the population of Bahrain at 2.1-2.9% versus the sickle cell trait frequency of 11.2-13.8%[4,5,6], the S-beta-thal genotype is expected to contribute a minority of patients having SCD in contrast with the vast majority of patients expected to have the sickle cell anemia type (SS genotype).

Our findings in this report revealed three distinct phenotype-based S-beta-thal disease in Bahrain with underlying 5 different SCD genotypes that are thoroughly described in this manuscript. The phenotype of these various S-beta-thal disorders are dictated primarily by the underlying beta-thal mutations: severe, moderate or mild/silent beta-thalassemia mutations. The first form of S-beta-thal is the severe type which is contributed mainly by two different underlying genotypes: the coinheritance of the beta-thal mutation at Cd39 (C-T) [c.118C>T; p.Gln40Ter] along with the sickle cell mutation [c.20A>T; p.Glu7Val] (n=18/31; 58%); or the 25 bp del [c.93-22_95del] along with the sickle cell mutation (n=13/31; 42%). These two beta-thal mutations are, indeed, the most common beta-thalassemia mutations in the population of Bahrain[18]. The remaining number of patients in this severe category of S-beta-thal, (n=6), are caused by the coinheritance of other minor beta-thal mutations uncovered in Bahrain, either ?° or severe ?$^{+}$, along with the sickle cell mutation in trans [no further elaboration on these 6 SCD genotypes in this report].

Curiously we uncovered diversion of two clinical phenotype presentation in S/Cd 39 versus S/25 bp del genotypes despite the fact that both Cd 39 C-T and the 25 bp del mutations are of the ?°-thal type mutations. The genotype of S/Cd39 behave, generally, same as the severe state of SCD with more frequent VOCs, hospitalizations, and the need for infrequent blood transfusion (Table 3). In contrast, the S/25 bp del genotype presented generally with a less severe clinical profile including less frequent VOCs, hospitalization, and the need for blood transfusion (Table 3). The relatively lower severity uncovered in S/25 bp del genotype might be attributed, at least partially, to the average higher level of HbF in S/25 bp del at 19.4 ± 8.3% versus S/Cd 39 at mean level of 16.2 ± 7.7% (Tables 1 and 2). Indeed we uncovered previously that the 25 bp del mutation in Bahrain is in linkage disequilibrium (LD) with two different beta-globin haplotypes (haplotypes I and IX); whereas Cd 39 mutation found solely on beta-globin haplotype I[19]. The beta-globin haplotype IX is, indeed, harboring the C-T polymorphism in the promotor region of the G-gamma globin (*HBG2*) gene at nucleotide -158 upstream of the transcription initiation site of this gene (i.e., the *Xmn* I site; rs7482144) as published before[20]. This polymorphism was, indeed, found in linkage disequilibrium as well with the Arab-Indian (AI) and Senegal (SEN) HbS haplotypes which are associated in turn with higher HbF expression and milder clinical presentation[21,22]. In contrast the other *Xmn* I–negative African HbS haplotypes (i.e., the Benin, Bantu (or CAR) and Cameroon haplotypes) are associated with lower levels of HbF expression and more severe clinical presentation[21,22]. Previous work showed that the vast majority of HbS haplotypes in Bahrain is indeed of the Arab-Indian HbS haplotype[8,19]. Same sickle haplotype background was uncovered for SS and S-beta thal patients from the geographically close-by Eastern province of Saudi Arabia reflecting a genetic homogeneity with expected similar clinical presentation in these patients[23]. In contrast, in one study from Brazil the S-beta-thal disease (with underlying ?° or severe ?$^{+}$-mutations) revealed a much more severe presentation of the disease[16]. Presumably the sickle cell haplotype is a major underlying difference between our S-beta-thal patients and the patients found in Brazil. In Brazil the severe African HbS haplotypes are mostly encountered, specifically the Bantu and Benin haplotypes[24], whereas the less severe Arab-Indian HbS haplotype is mostly encountered in our region as mentioned above.

The second type of S-beta-thal disease in Bahrain attributed to the coinheritance of nt -88 (C-A) with the sickle cell mutation giving rise to a moderate phenotype of sickle cell disease. Patients with this genotype rarely have vaso-occlusive events or require hospital admission (Table 3), and presented with modest, albeit distinct, hematological changes especially in the Hb electrophoresis profile (Table 4, Figure 1).

The third type of SCD contributed by two different genotypes with underlying beta-thal mutations of the silent/mild forms, i.e., nt -101 (C-T) [c.-151C>T] and nt -71 (C-T) [c.-121C>T], that either co-inherited with the sickle cell mutation in trans. Both of these SCD genotypes result in asymptomatic sickle cell disease condition that resemble to a large extent, hematologically and clinically, the sickle cell carrier state (SCT) (Tables 3,5,6).

This study has encountered a number of limitations. First, this was a retrospective study and much of the clinical details was not available in the electronic health record (EHR). It would more informative if we have more detailed EHR that enlist subtle clinical outcomes, for instance the extent of spleen involvement/enlargement (splenomegaly). Second, this study would be more comprehensive if the findings presented, especially for the moderate and mild forms of S-beta-thal, are followed up for longer term, including aged patients, in order to uncover time-limited subtle complications. Finally, the study would be more informative if other rare SCD genotypes (for instance the excluded 6 SCD genotypes) are uncovered with a significant number of cases that could be thoroughly investigated and compared with the reported genotypes in this study.

In summary we report in this manuscript the various type of S-beta-thalassemia disease in Bahrain and correlate the underlying genotypes with the different phenotype presentations. Overall, our findings indicate a quite heterogeneous clinical presentation of the S-beta-thal disease in Bahrain ranging from asymptomatic and healthy individuals to the typical severe form of SCD that are largely attributable to the type of the underlying ??thalassemia mutation in relevant genotypes. This study would be an invaluable contribution for understanding the versatile nature of sickle cell disease in Bahrain and contribute important aspects for the SCD screening, premarital services and the overall clinical care of SCD in the population of Bahrain.

CONCLUSION

In conclusion we provided in this manuscript an in-depth analysis of the various type of sickle cell-beta-thalassemia disease in Bahrain on the molecular and phenotypic level. This would be invaluable for better understanding of the disease and, eventually, rational management of patients afflicted with these disorders. Our findings would also add an invaluable information for a cohesive and rational genetic counseling for patients and their families based on scientifically validated data.

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