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Sickle Cell Disease in Jordan: The Experience of a Major Referral Center

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ABSTRACT

Introduction: Sickle cell disorders are the most frequently encountered hemoglobin variants in Jordan. Both alpha and beta thalassemias are also prevalent in this population. However, studies on the interaction between these hemoglobin disorders are lacking. **Aim:** To determine the genotypes responsible for Sickle cell disease in Jordan, by retrospectively reviewing the data from a major referral center in the country's capital. **Methods:** A total 29,712 peripheral blood samples referred and investigated for hemoglobinopathies over a 10-year period at Princess Iman Center at Amman, Jordan were retrospectively reviewed. In addition to full blood counts, high performance liquid chromatography, those who were identified with sickle cell hemoglobin were studied using polymerase chain reaction and reverse hybridization to determine the various sickle cell disease genotypes. **Results:** Out of the (29,712) blood samples, 450 were sickle cell trait, while 216 had sickle cell disease. Of the latter: 120 were found to be cases of Sickle cell anemia (Hb SS), 66 were compound heterozygous for Sickle cell and a beta thalassemia mutation (Sickle/ β -thalassemia), while 30 had concomitant alpha thalassemia (HbSS/ α -thalassemia). The most frequent genotype associated with sickle/ β -thalassemia was HbS/ IVS-110 (G>A), followed by Hb S/ IVS-I-6 (T>C), HbS/IVS-II-745 (C>G) and HbS/ IVS-II-1 (G>A). While the most frequent alpha genotype detected in HbSS/ α -thalassemia samples was ($-\alpha^{3.7}/\alpha$) followed by ($-\alpha^{3.7}/-\alpha^{3.7}$). Hb SS patients had the severest hematological phenotype compared to those with sickle/ β -thalassemia and sickle/ α -thalassemia. Furthermore, within the sickle/ β -thalassemia subgroup the least severe hematological phenotype was encountered in HbS/IVS-1-6 (T>C), while the most severe in HbS/IVS-II-1 (G>A) genotype. **Conclusion:** The most frequent Sickle cell disease genotype in Jordanians is Sickle cell anemia (HbSS), followed by Sickle/ β -thalassemia and least frequent is HbSS/ α -thalassemia. The concomitant identified thalassemia mutations were consistent with their spectrum among the Jordanian population.

Keywords: Sickle cell anemia, Sickle/beta thalassemia. IVS-1-110, IVS-II-745, $-\alpha^{3.7}$

1. INTRODUCTION

The sickle cell hemoglobin (HbS) is the most common variant hemoglobin, resulting from a single amino acid substitution of valine for glutamic acid at position 6 of the β -globin chain of hemoglobin (1). Sickle cell disease (SCD) encompasses a group of symptomatic disorders, due either to homozygosity to sickle cell gene (HbSS, sickle cell anemia), compound heterozygosity to sickle cell and β -thalassemia gene (Sickle/ β thalassemia), or to sickle cell and other β -chain structural variants (e.g. Hb SD, Hb SC, etc) (2).

Earlier studies have documented that sickle cell gene prevalence rates range from 0.44 to 6.0% in various areas of Jordan (3-6), while β -thalassemia and α -thalassemia prevalence rates range from 3.0-5.9% and 2.3-3.5% respectively (3-5, 7). However, studies on the molecular basis of SCD and its association with thalassemia are sparse. Accordingly, the current study was initiated based on data from a large referral center in Jordan.

2. AIM

To determine the genotypes responsible for SCD in Jordan, by retrospectively reviewing the data from a major referral center in the country's capital.

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3. METHODS

A total of 29,712 samples were referred to Princess Iman research and laboratory sciences center in the period between for 2009 to 2018 to assess for hemoglobinopathies. All samples were subjected to full blood counts and high-performance liquid chromatography using Bio-Rad Variant II instrument (BioRad, USA). Samples with hemoglobin S peak identified by HPLC, had further confirmation of their disorder by a standard sickling test.

Genomic DNA was extracted using the DNA extraction kit (Promega- USA). The extracted DNA was thereafter subjected to multiplex PCR and reverse hybridization to detect the sickle cell (codon 6[A>T] and Hb C codon 6 [G>A] mutations, and 20 β -thalassemia mutations using β -globin StripAssay according to the manufacturer instructions (ViennaLab Diagnostics GmbH, Austria). The 20 β -thalassemia mutations screened for were: -101[C>T], -87[C>G], -30[T>A], codon 5[-CT], codon 6[-A], codon 8 [-AA], codon8/9[+G], codon 15[TGG>TGA], codon 27 [G>A], IVS1.1[G>A], IVS1.5 [G>C], IVS 1.6[T>C], IVS1.110[G>A], IVS1.116[T>G], IVS 1.130[G>C], codon 39 [C>T], codon 44 [-C], IVS 2.1[G>A], IVS 2.745[C>G], IVS 2.848[C>G].

In some patients with Hb SS and with unexplained hypochromia, Alpha thalassemia was screened for by using α -globin StripAssay according to manufacturer's instruction (ViennaLab Diagnostics, Austria). The 21 mutations screened for were namely: α -globin mutations, including two single gene deletions (-a3.7; -a4.2), five double gene deletions w-MED; -SEA; -THAI; -FIL; - (a) 20.5x, anti-3.7 gene triplication, two point mutations in the $\alpha 1$ gene (cd 14 G)A; Hb Adana) and 11 point mutations in the $\alpha 2$ gene (initiation cd T)C; cd 19 -G; IVS1 -5nt; cd 59 G)A; Hb Quong Sze; Hb Constant Spring; Hb Icaria; Hb Pakse; Hb Koya Dora; polyA-1; polyA-2.

Statistical analysis was analyzed using the IBM SPSS® software. Comparison of means between groups was determined using the independent t-test and P-values of < 0.05 were interpreted as significant.

This study was approved by the Ethical committee at the Jordan Royal Medical Services.

4. RESULTS

Out of 29,712 cases, there were 16,345 females (54%) and 13,367 males (46%), with an age range of 1-7 years (median 4 years). Referrals came from different peripheral hospitals and variable geographical areas in Jordan.

The 29,721 samples included 795 samples with detectable hemoglobin structural variants by HPLC, including 666 where that variant was HbS. The latter included 450 with sickle cell trait, and 216 SCD. Based on the blood counts and HPLC results the latter group was studied further to sub-classify it into SCA and sickle/ β -thalassemia using molecular techniques. Molecular studies identified 66 patients as sickle/ β -thalassemia, and characterized their β -thalassemia mutations as shown in Table 1. The beta thalassemia mutations encountered were IVS 1-110 (G>A) in 25 cases, IVS 1-6 (T>C) in 17, IVS2.745 (C>G) in 13 and IVS2.1 (G>A) in another 12

Genotype	Number (%)
Codon 6 (A>T)/IVS-1-110[G>A]	25 (37.9)
Codon 6 (A>T)/IVS-1-6[T>C]	17 (25.8)
Codon 6 (A>T)/IVS -2-745[C>G]	13 (19.7)
Codon 6 (A>T)/IVS-11-1[G>A]	11 (16.7)

Table 1. The genotypes and number of Sickle/beta thalassemia samples

Hb parameters	HbS [A>T]/ IVS1-6 [T>C]	HbS [A>T]/ IVS1-110 [G>A]	HbS [A>T]/ IVS- II-745[G>C]	HbS [A>T]/ IVS-II-1 [G>A]
Hb (g/d)	10.07±0.456	9.49 ±0.258	8.53±0.487	7.51±0.403
MCV (fl)	71.7 ±1.094	70.1±3.043	69±2.121	67±1.819
MCH (pg)	24.75 ±1.194	22.48 ±1.113	23.05 ±1.222	22.04 ±0.916
Hb A ₂ (%)	3.8±0.128	4.5 ±0.609	4.7±0.510	5.1 ±0.360
Hb F (%)	7.1±0.379	9.0 ±2.132	12± 0.99	13 ±0.634
Hb S (%)	65.05±3.295	68.28 ±3.085	71.23± 1.028	74.13 ±1.801

Table 2. Red cell indices in the four beta thalassemia mutations associated with HbSS.

Hb parameters	HbSS (SCA) (No. 120)	HbS/ β -thalassemia (No. 66)	HbSS/ α thalassemia (No. 30)
Hb (g/d)	7.6 ± 1.097	9.12 ±0.971	8.34±0.267
MCV (fl)	85.92 ± 5.605	69.77 ±2.727	69.0±6.377
MCH (pg)	26.11±0.727	23.11 ±1.513	23.40±0.844
Hb A ₂ (%)	3.45 ± 0.790	4.45 ±0.637	3.88±0.248
Hb F (%)	13.67 ± 7.699	9.79 ± 2.638	15.28±2.102
Hb S %	71.33 ± 12.835	69.0 ±4.074	75.5±5.720

Table 3. The Red cell indices in the three genotypes of Sickle cell disease.

cases. Other β -chain structural variants were not found in our series. Of the remaining 150 SCD patients, 30 patients had unexplained hypochromia, thus a presumed diagnosis of HbSS/alpha thalassemia. The latter were further studied to determine the associated alpha thalassemia mutations, and it was found that 9 were HbSS with concomitant $-\alpha^{3.7}/-\alpha^{3.7}$, while 21 were HbSS with concomitant $-\alpha^{3.7}/\alpha$. Non-deletional mutations were not detected in our examined samples.

The hematological parameters associated with HbSS and with Sickle/ β -thalassemia are outlined in Table 2. When hematological parameters in patients with HbS/IVS1-6 were compared with the other three S/ β -thalassemia mutations, it was found that the former had the highest Hb and MCH (all with p values of <0.001), as well as the highest mean MCV (P=0.001 for HbS /IVS-2-745, 0.041 for IVS-1-110 and <0.001 for IVS-2-1). On the other hand, the mean Hb F, Hb A₂, and Hb S were the least in samples with HbS/ IVS 1-6 compared to all each of the other three S/ β mutations (all at P <0.001). On the other hand, comparing the hematological parameters in patients with HbS/IVS-2-1 with the other three S/ β -thalassemia mutations, it was found that the former had the lowest mean Hb, MCH and MCV, and the highest mean Hb F, HbA₂ and Hb S (all at P <0.001) compared to the other three mutations.

The hematological parameters of HbSS (SCA), HbS/ β -thalassemia and Hb SS/ α -thalassemia are outlined in Table 3. Comparing samples of HbSS with

HbS/ β -thalassemia, it was found that the later had significantly higher mean Hb and Hb A₂ (both at P <0.001) and significantly lower mean MCV, MCH and Hb F (all with P <0.001). There was no significance difference in mean Hb S (P value=0.174). Whereas comparing samples of HbSS with Hb SS/ α -thalassemia it was found that the later had significantly higher mean Hb (P=0.042) and lower mean MCV and MCH (both at P <0.001). No significant differences were found in the mean Hb S, Hb F, or Hb A₂ (P values of 0.325, 0.520, and 0.101 respectively).

5. DISCUSSION

The current study is the first to address the issue of molecular characterization of sickle cell disease in Jordan. The high prevalence rates of both sickle cell gene and thalassemia among Jordanians, makes the probability of their concomitant inheritance an important cause (entity) of SCD in this part of the world.

IVS-1-110 is a Mediterranean mutation which is the most common β -thalassemia mutation in many Arab countries including Egypt, Iraq, Lebanon, Syria, Gaza Strip, and Saudi Arabia as well as Jordan (8-12). Thus, the observation that it is the most common β -thalassemia associated with sickle cell gene in the current study is quite expected.

IVS-I-6 is another frequent mutation in the Eastern Mediterranean region with highest rates in Palestinian West bank, but has also been reported in high frequencies in some Egyptian studies, Lebanon as well as Jordan (8, 10, 12, 13). This consistent with the current study where it was the second most frequent encountered mutation in Jordanian Sickle/ β -thalassemia.

IVS-II-745 is a mutation which has its highest frequency in Jordan (8), where it may have originated has also been reported though in lower frequencies in Egypt and Morocco (13, 14). This explains its high frequency among our HbS/ β -thalassemia patients.

IVS-II-1 is a common Eastern Mediterranean mutation, which has its highest frequencies in Iran, some Arabian Gulf countries and northern Iraq (8, 15-19), but is also frequent in neighboring Arab countries like central Iraq as well as Jordan where it is the second most frequent mutation following IVS-1-110 in these two countries (8, 20). This explains its association with sickle cell gene in the current study.

The association of 20% of Hb SS with rightward α -thalassemia deletion ($-\alpha^{3.7}$) could be explained by the fact that alpha thalassemia has been reported in 2.26-3.5% of Jordanians (4, 5, 7), with ($-\alpha^{3.7}$) deletion being the most frequently encountered mutation in this population and in Arabs in general (20-22). However non deletional mutations were not found in our study.

The hematological findings in patients with sickle cell anemia (HbSS) clearly show a more severe anemia, with normochromic indices as compared to those Sickle / β thalassemia, which is consistent with the literature (23, 24). Moreover, the mildest phenotype among the four HbS/ β -thalassemia genotypes, as demonstrated by higher mean hemoglobin and less reduction of mean

MCV/MCH, is the HbS/IVS-I-6, the latter is a β^+ mild thalassemia mutation. The most severe phenotype, on the other hand, is that encountered among those with HbS/ IVS-11-1, as demonstrated by higher mean Hb A₂ and Hb S, the latter being a severe β^0 mutation (23, 25). On the other hand, patients with Hb SS/ α -thalassemia show milder disease than HbSS patients as demonstrated by higher mean Hb (26).

6. CONCLUSION

The current study documented that while SCD is mainly due to homozygosity to sickle cell gene (SCA), however genotypes due concomitant inheritance of α or β -thalassemia mutations, tend to modify phenotype and constitute nearly 44% of SCD in this population.

- **Abbreviations:** Sickle cell hemoglobin = HbS, Sickle cell disease = SCD, Sickle cell anemia = HbSS.
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