

Heterogeneity of β s gene haplotypes in patients with sickle cell disease (SCD) in Oman: A review of relevant publications

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Abstract. Sickle cell disease (SCD), caused by a mutation in the β -globin gene HBB, is widely distributed in malaria endemic regions. The prevalence of sickle cell trait and disease reaches up to 4.8–6% and 0.2% respectively, which is the highest among the Arab Gulf states. Omani population represents a variability of HbS genotype combinations with other Hb genotypes modify the clinical severity of the disease. The most prevalent sickling abnormality in Oman is Hb S/S (SCA) followed by Hb S/ β -thalassemia. Omani children with SCD with high Hb F level had less severe disease. More than two-thirds of SCD cases were running a mild course of the disease due to the high prevalence of α -thalassemia trait. The severity index has been correlated with the early age of presentation, the absence of α -thalassemia trait and the lower level of HbF as well as to the existence of different β -globin gene haplotypes. S/ β^0 presented with the same clinical severity of S/S while those with S/ β^+ had some splenic function into adulthood and were more prone to splenic sequestration. The unique existence of HbS-Oman (a severe variant of sickle hemoglobinopathy) markedly increased the severity of the disease. Compound heterozygotes HbS-Oman resulted in very severe clinical manifestations with transfusion-dependency and hypersplenism early in life. This paper summarizes and reviews β s gene haplotypes in patients with sickle cell anemia (SCA) in Oman. (www.actabiomedica.it)

Key words: Sickle cell disease, Hb SS, genotype, phenotype, severity, HbF, Hb S β -thalassemia, Oman

Introduction

The Sultanate of Oman is an Arab Gulf country in Western Asia. It is situated on the south-eastern coast of the Arabian Peninsula. Oman shares land borders with Saudi Arabia, United Arab Emirates and Yemen. The population of the Arabian Peninsula is known to have a high incidence of hemoglobinopathies, including α - and β -thalassemia, Hb S, Hb C, Hb E, Hb D and Hb S^{oman} (1). Therefore, the Omani population with sickle cell disease (SCD) represents a variability

of homozygous SS patients (>80%) and combination Hb S variant with other abnormal Hbs or HBB thalassemia variants (β^0 , β^+ , $\delta\beta$) that can present with different phenotypic severity.

Many studies support the idea that genetic heterogeneity is related to major discrepancy in hematological parameters and clinical severity of presentations. Studying the β s gene haplotypes in patients with SCD in Oman (basically haplotypes of β s gene) can improve prediction of the prognosis and occurrence of different complications.

Aims and methods

In this review, we studied and evaluated all published research papers (PubMed, Google Scholar and Scopus) on SCD in Oman in the past 20 years and tried to analyse the heterogeneity of β s gene haplotypes in Omani patients. Research articles which were published during the period from 2000 till 2021 were reviewed and evaluated.

Results of the review

Historically, Oman was the main trading port of the Arabian Gulf region. Its high trade activities resulted in a mixed social and ethnic background of the Omani people. Zanzibar, Pakistan, and parts of Iran were classified as malaria-endemic areas as well as some parts of the Arabian Peninsula (Malaria Belt). Malaria is one of the main reasons for the occurrence of SCD, thalassemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency and other abnormal hemoglobin as well as erythrocyte defects which are the most common Mendelian diseases. Different populations have evolved different genetic variants to protect against malaria (2,3). It is known that there has been positive natural selection for hemoglobin S and C in humans despite negative health effects, due to its beneficial role in malaria resistance. However, it is not known if there has been natural selection for hemoglobin E (HbE), which is a common variant in Southeast Asia (4).

In 1995, a national register of symptomatic hemoglobinopathies in Oman identified 1.757 β -cases of homozygous sickle cell anemia (SCA) and 243 cases of β -thalassemia major in a population of 1.5 million in 1995. 10% had for sickle cell trait and 4% had β -thalassemia trait. They reported the birth prevalence of symptomatic haemoglobinopathies in 23 Omani tribes through screening of a national register, as 1 in 323 live births or 3.1 per 1,000 live births during 1989-1992, which included 2.7 per 1.000 live births of homozygous SCD (5).

In 2001, Al-Riyami et al. (6), performed a nationwide survey representative sample of 6.103 Omani households and blood samples from 6.342 children aged 0-5 years. Countrywide prevalence rates for the sickle-

cell and β -thalassemia traits were estimated to be 5.8% and 2.2%, respectively, with no significant gender differences. The prevalence of SCD was 0.2% and homozygous β -thalassemia was 0.07%. The prevalence of sickle cell trait was relatively higher than those reported from Saudi Arabia (1.2%) and United Arab Emirates (1.9%). Nowadays, in our hematology outpatient clinics, we encounter considerably high number of infants diagnosed with different types of SCD in Oman. Unfortunately, we don't have a national registry for SCD yet.

In 2010, Alkindi et al. (7), studied consecutive cord blood samples from a total of 7,837 neonates for complete blood counts and for hemoglobin (Hb) profile by high performance liquid chromatography (HPLC). They observed that the overall incidence sickle cell trait was 4.8%, sickle cell disease 0.3%, β -thal trait was 2.6%, Hb E trait was 0.9%, Hb D trait was 0.8%, and homozygous beta-thal) was 0.08%. In addition, cases of HbS Oman, a variant of HbS were identified in a few families.

Clinical variations in SCA presentation were largely related to the presence of different β s-globin gene haplotypes identified during molecular studies. Daar et al. (1), demonstrated the multicentric origin of the sickle mutation in Northern Oman. They reported the coexistence of three major haplotypes coexist: 52.1% Benin (typical and atypical), 26.7% Arab-India, and 21.4% Bantu. The distribution of haplotypes was in excellent agreement with the historical record, which established clear ancient contacts between Oman and sub-Saharan west Africa which explained the presence of the Benin haplotype. Contacts with Iraq, Iran, present-day Pakistan, and India explained the presence of the Arab-India haplotype. The more recent contacts with East Africa (Zanzibar/ Mombasa) explained the presence of the Bantu haplotype (1).

Daar et al. (1), reported that most of the patients (61.2%) were homozygous sickle cell anemia followed by double heterozygous types mainly sickle cell β -thalassemia. In 52 patients with SS genotypes, the Arab-India haplotype in the homozygote form was associated with higher levels of HbF as compared to the Benin and Bantu haplotypes. The Arab-India haplotype in the heterozygous form had an average HbF level lower than homozygous cases of this haplotype. Other investigators found that the African haplotypes

were predominant (68.5%) and reported that 80% of those screened for α -globin gene mutations were heterozygous or homozygous for α -thalassemia. They reported that the presence of alpha thalassemia gene mutation and high Hb F levels were important factors modifying the clinical severity of the disease (8-10).

In a relatively recent study, Hassan et al. (11) found 11 haplotype combinations differently distributed in Oman and stated that the Asian/Asian haplotype was the most prominent while Benin/Benin came the second in rank. The higher percentages of Benin haplotype stated in Daar et al. (1) study might be due to their selection of patients who were attending the Sultan Qaboos University Hospital which covers only one region (Dhakhiliya region). The Benin haplotype has been observed to be present at a high rate in this region (1,5,11).

Hassan et al. (11,12) suggested that the Asian haplotype was associated with the highest HbF levels, fewer hospitalizations and painful episodes and acute chest syndrome. They confirmed that the CAR haplotype whether homozygous or combined heterozygous was associated with the lowest HbF level and the highest incidence of organ damage and renal failure.

Al-Lamki et al. (13) and Saraf et al. (14) reported that the absence of co-inheriting α -thalassemia, and low hemoglobin F levels was associated with more hemolysis, and lower hemoglobin oxygen saturations.

In Oman, the most prevalent SCD is S/S followed by S/ β thalassemia. Sick cell β -thalassemia (S/ β -thalassemia) is a condition, which results from the coinheritance of a sickle cell gene and a β -thalassemia gene. The clinical phenotype depends on the type of β -thalassemia gene (β^+ or β^0) inherited. S/ β^0 runs almost the same clinical course of S/S (10). As previously described patients with the S β^0 phenotype had a higher degree of hematological involvement in comparison to S β^+ patients, with lower hemoglobin levels, and signs of more intense chronic hemolysis. S β^0 patients had decreased body mass index and lower bone mineral density. The degree of bone damage correlated to lower body mass index (BMI) and hemoglobin levels, as well as monocytosis and elevated lactate dehydrogenase, possibly reflecting the effects of hemolysis and inflammation upon bone metabolism and body constitution (15). Children with sickle cell- β^+ thal-

semia may have preserved some splenic function into adulthood. Their spleen might remain till late adulthood, and they rarely have auto-splenectomy. They are more prone to splenic sequestration than other genotypes of SCD (16,17).

Trying to find a molecular explanation for the different phenotypes seen within similar basic haplotypes, sub-haplotypes were determined by looking at a total of 42 SNPs in 125 homozygous HbS patients. Out of the 42 SNPs, only 15 SNPs were found modifying the 11 identified haplotypes. However, no sub-haplotypes were found to be associated with a specific haplotype except for the CAR/OmanI that showed nucleotide variations at the G-g(SNP1) (SNP position: 5232979-5232984) located in the G-g promoter (11,12). However, both Daar et al. (1) and Hassan et al. (11,12), suggested that neither the haplotype or sub-haplotype nor the HbF alone appeared to be fully responsible for the variable clinical phenotypes.

Adding to the array of the disease in Oman is the unique existence of HbS-Oman. Hb S-Oman is a severe β s gene variant of sickle hemoglobinopathy that results from 2 simultaneous mutations in the same β -globin chain. The first is the classic Bs mutation (B6 Glu Val) and the second is in position 121 (B121 Glu Lys) (3).

HbS-Oman was first described by Langdown et al. (18) and is one of these rarer genotypes. It was named because of its high prevalence amongst individuals of the Omani population. The polymerization and sickling properties of HbS-Oman are considerably greater than that of HbS and it represents one of the "super sickling" forms of Hb. Accordingly, heterozygotes of Hb A/S-Oman genotype with HbS-Oman levels of 20% or less present with some of the clinical complications of SCD. This phenotypic dominance of the sickle mutation is shared only with HbS-Antilles, which has a further valine to isoleucine substitution at the b23 residue, additionally to the standard to sickle b6 glutamic acid-valine. By comparison, sickle cell trait individuals (Hb A/S genotype), with red cell levels of Hb S of about 40%, are usually silent carriers and their red cells seldom sickle in vitro. However, SCD patients of the HbS-C genotype, who share a similar, though milder, clinical course with those of the Hb S/S genotype, have Hb S levels of around 50% (19-22).

Compound heterozygotes of Hb-S-S^{Oman} were identified. These compound heterozygote patients run a very severe clinical course like transfusion-dependent thalassemia major with hypersplenism early in life. Hypersplenism was not controlled, solely, by hypertransfusion and needed both splenectomy at the age of one year and bone marrow transplantation in the second year of life (3,15).

Wali et al. (3) reported six patients with compound heterozygosity for Hb-S-S^{Oman}, two patients underwent bone marrow transplantation (BMT) as they had extremely severe course of the disease. Fifty-six patients with Hb S^{Oman} heterozygosity were evaluated, their clinical features ranged from being asymptomatic to severe course of the disease. Patients with Heterozygous HbS Oman and compound heterozygous HbS-S

Oman had lower mean Hb, MCV, MCH and higher RDW values compared to SCD patients (21).

Al Balushi et al. (19), analyzed the clinical profile and red cell properties of 29 further cases of HbA/S-Oman individuals. Levels of sickling were considerable and could be elicited with levels of HbS-Oman as low as 4% in fully deoxygenated red cells. Considerable numbers of sickled cells were present at arterial O₂ tensions. Currently, we have more than 70 carriers of Hb-S^{Oman}, and 7 cases (Compound heterozygotes S-S^{Oman}).

The rare form of sickle cell disease, which is Hb SD, and several variants of HbD such as Hb D Punjab, Hb D Iran, Hb D Ibadan, and Hb D Bushman have been noted to co-inherit with HbS. Except for Hb D Punjab, compound heterozygous states of HbS with

Table 1. Summary of studies on the heterogeneity of β s gene haplotypes in patients with Sickle cell disease (SCD) in Oman.

Author	Year of publication	Type of study	Numbers of patients	Comments
Daar S, et al. (1)	2000	Cross Sectional	94	Authors reported the coexistence of three major haplotypes: 52.1% Benin (typical and atypical), 26.7% Arab-India, and 21.4% Bantu. 61.2% were homozygous sickle cell anemia followed by double heterozygous types mainly sickle cell β -thalassemia.
Al-Lamki Z, et al. (13)	2000	Retrospective	375	High Hb F level is one of the factors decreasing the clinical severity of the disease.
Al Riyami AA, et al. (9)	2001	Cross Sectional	6,342	Nationwide prevalence rates for the sickle-cell and β -thalassemia traits were estimated to be 5.8% and 2.2% respectively. The prevalence of SCD was 0.2% and homozygous β -thalassemia was 0.07%. SCD prevalence was the highest among Arab Gulf states.
Wali Y, et al. (3)	2002, 2017	Cross Sectional	6	Six patients with compound heterozygosity for Hb-S-S ^{Oman} , two patients underwent bone marrow transplantation as they had extremely severe course of the disease. > 60% Omani children with SCD had preserved splenic function.
Knox-Macaulay HH, et al. (22)	2007	Cross Sectional	12	12 patients with compound heterozygous Hb SE from six unrelated Arab families were studied. 50% of them were asymptomatic throughout the study. However, sickling-related complications occurred in the rest (6/12) including acute chest syndrome (1/12), severe vaso-occlusive skeletal pain (2/12)
Alkindi S, et al. (7)	2010	Prospective	7,837	Through neonatal screening, the incidence of sickle cell trait was 4.8%, sickle cell disease 0.3%, β -thal trait was 2.6%, Hb E trait was 0.9%, Hb D trait was 0.8%, and homozygous β -thal was 0.08%.
Hassan S, et al. (11,12)	2010, 2015	Cross Sectional	125	11 haplotypes of SCD combinations were differently distributed in Oman. The Asian/Asian haplotype was the most prominent while Benin/Benin came second. Asian haplotype was associated with the highest HbF levels, and fewer hospitalizations and painful episodes.
Al Balushi HWM, et al. (19)	2017	Prospective	29	In 29 cases of HbA/S-Oman., levels of sickling were considerable and could be elicited with levels of HbS-Oman as low as 4% in fully deoxygenated red cells.

HbD variants were clinically innocuous. Studies have shown that the clinical presentations of HbSD-Punjab mimicked severe form of sickle cell anemia (23). In Oman, Hb S-D patients behave exactly like Hb S-S disease with almost similar severity (19).

In summary, in Oman, the prevalence of sickle cell trait reaches up to 4.8–6% which is the highest between the Arab gulf states. Omani population represents a variability of Hb S genotype combinations with other Hb genotypes that can present with different phenotypic severity. The most prevalent SCD is S/S followed by S/ β -thalassemia. The clinical variations in SCD presentation were largely linked to the presence of different β -globin gene haplotypes identified during molecular studies. S/ β^0 runs almost the same clinical course of S/S. Children with S/ β^+ thalassemia appears to be more prone to splenic sequestration compared to other genotypes of SCD. Co-inheriting of α -thalassemia, and higher hemoglobin F levels was associated with less hemolysis and milder course. The unique existence of HbS-Oman markedly increases the severity of the disease. Compound heterozygotes HbS-Oman result in a very severe clinical manifestations with transfusion-dependency and hypersplenism early in life. Identification of additional genetic modifiers can improve prediction of the prognosis and complications in SCD. A summary of research performed on the heterogeneity of SCD in Oman and its associations is presented in table 1.

Conflicts of interest. Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Funding. None.

Author Contributions: Study conception and design: YW. Data collection: NA, YW. Analysis and interpretation of results: YW, AS. Draft manuscript preparation: NA, AA. Draft editing and preparation of summary table: YW, AS, DK. All authors reviewed the results and approved the final version of the manuscript

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Received: 18 April 2021

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