Dr. Aida El Alginawi / Afr.J.Bio.Sc. 4(4) (2022).439-445 ISSN: 2663-2187

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Comprehensive Genetic and Phenotypic Analysis of Hemoglobinopathies in Multi-Ethnic Populations: Investigating the Clinical, Molecular, and Epidemiological Aspects of Sickle Cell Disease and Thalassemia

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Article History
Volume 4, Issue 4, Dec 2022
Received: 15 Oct 2022
Accepted: 25 Nov 2022
Published: 25 Dec 2022

Abstract

Background: Sickle cell disease (SCD) and thalassemia are prevalent inherited hemoglobinopathies with varying clinical severity. Genetic modifiers, such as alpha-thalassemia and fetal hemoglobin (HbF) levels, play a crucial role in influencing the clinical outcomes of these disorders. **Objectives**: To perform a comprehensive genetic and phenotypic analysis of SCD and thalassemia in a multi-ethnic cohort, exploring the impact of genetic mutations and modifiers on clinical severity. **Methods:** A cross-sectional study was conducted on 55 patients, including 30 with SCD and 25 with thalassemia. Clinical data were collected, and genetic analysis was performed to identify common mutations and genetic modifiers, such as alpha-thalassemia and HbF levels. Statistical analyses were conducted to assess the relationships between genetic factors and clinical outcomes. **Results:** In SCD, 85% of patients were homozygous for HbSS, with 25% also having alpha-thalassemia and 40% exhibiting elevated HbF levels (>15%). These modifiers were associated with milder disease, fewer vaso-occlusive crises, and higher hemoglobin levels (mean 9.1 g/dL). Thalassemia patients with β0 mutations experienced severe anemia (hemoglobin <7 g/dL) and were transfusion-dependent, while those with β+ mutations had moderate anemia and required less frequent transfusions. **Conclusion:** The study highlights the significant role of genetic modifiers in determining the severity of SCD and thalassemia. Alpha-thalassemia and elevated HbF levels were associated with improved clinical outcomes in SCD, while the type of beta-globin mutation influenced the severity of thalassemia. Understanding these modifiers is crucial for genotype-guided management and personalized treatment strategies.

1. **Introduction**

Hemoglobinopathies, including Sickle Cell Disease (SCD) and Thalassemia, represent a group of inherited disorders characterized by abnormalities in hemoglobin, the protein in red blood cells responsible for carrying oxygen throughout the body. These genetic conditions are particularly prevalent in regions such as sub-Saharan Africa, the Middle East, India, Southeast Asia, and the Mediterranean, where they contribute significantly to global health burdens [1] . Despite advances in medical research, these diseases continue to pose major challenges due to their complex clinical manifestations and varying severity across different populations[2]. The comprehensive study of the genetic and phenotypic variations of hemoglobinopathies, alongside the clinical, molecular, and epidemiological factors influencing Sickle Cell Disease and Thalassemia, is crucial for improving our understanding of these conditions [3]. Sickle Cell Disease is caused by a mutation in the β-globin gene that leads to the production of abnormal hemoglobin known as hemoglobin S (HbS). Under conditions of low oxygen, HbS tends to polymerize, causing red blood cells to become rigid and sickle-shaped. These sickle cells obstruct blood flow, leading to painful vaso-occlusive crises, chronic hemolysis, and organ damage [4]. The severity of SCD varies significantly among individuals, with some experiencing frequent and life-threatening complications, while others have milder symptoms. This variation can be attributed to both genetic and environmental factors, including the co-inheritance of other genetic traits such as alpha-thalassemia or elevated levels of fetal hemoglobin (HbF), which can modulate disease severity [5]. Thalassemia, on the other hand, is a group of disorders that result from mutations in the genes responsible for producing the alpha or beta globin chains of hemoglobin. These mutations lead to an imbalance in globin chain production, causing ineffective erythropoiesis and chronic hemolytic anemia. Like SCD, the clinical presentation of Thalassemia ranges from mild to severe, depending on the type and number of mutations inherited. Individuals with severe forms of Thalassemia, such as beta-thalassemia major, often require lifelong blood transfusions and iron chelation therapy to manage the condition [6]. The genetic heterogeneity of Thalassemia is vast, with numerous mutations identified across different ethnic groups, further complicating diagnosis and management. This study, focusing on the comprehensive genetic and phenotypic analysis of hemoglobinopathies, aims to explore the clinical, molecular, and epidemiological aspects of Sickle Cell Disease and Thalassemia across multi-ethnic populations [7]. By investigating the genetic mutations underlying these disorders, as well as their phenotypic expressions, the research seeks to uncover the factors that contribute to disease variability. In addition to studying the genetic basis of these diseases, the study will also examine how environmental factors, healthcare access, and population demographics influence disease prevalence and outcomes [8]. Through this multi-faceted approach, the research aims to improve diagnostics, advance personalized treatment strategies, and inform public health policies targeted at populations affected by hemoglobinopathies [9].

Objective

The study's main objective is to find the comprehensive genetic and phenotypic analysis of hemoglobinopathies in multi-ethnic populations and investigate the clinical, molecular, and epidemiological aspects of sickle cell disease and thalassemia.

Methodology

This cross-sectional study was conducted at Saudia Arabia's various tertiary units and local city from 2021 to 2022. Data were collected from 55 patients.

Inclusion criteria:

1. A confirmed diagnosis of either Sickle Cell Disease or Thalassemia.
2. Age range between 18-65 years old.
3. Written informed consent to participate in the study.

Exclusion criteria:

1. Patients with other hematological disorders or co-morbidities that could confound the results.

Data Collection

Detailed clinical histories were obtained from medical records and patient interviews. Information collected included the frequency of complications (such as vaso-occlusive crises for SCD and transfusion requirements for Thalassemia), history of infections, treatment regimens, and organ function assessments. Physical examinations and laboratory tests were conducted to evaluate hemoglobin levels, liver and kidney function, and markers of hemolysis. Blood samples were collected from all 55 patients for DNA extraction and genetic analysis. The primary focus was on identifying mutations in the HBB gene responsible for Sickle Cell Disease and mutations in the HBA and HBB genes associated with Thalassemia. Genetic sequencing methods, including PCR (Polymerase Chain Reaction) and Sanger sequencing, were employed to confirm known mutations and detect novel genetic variations. The following primers were used to amplify the target gene regions:

- **HBB gene (SCD mutation):**
  - Forward Primer: 5'-CCTGTGGAGGAGAAGTCTGCCGTTA-3'
  - Reverse Primer: 5'-GAGTGGACAGATCCCCAAAGGACTC-3'
- **HBA gene (Thalassemia mutation):**
  - Forward Primer: 5'-TGGACCCAGAGGTTCTTTGAGTCAC-3'
  - Reverse Primer: 5'-AGACGGCTGGTTTAACTCCAGCCAC-3'

In addition, secondary genetic modifiers such as alpha-thalassemia and fetal hemoglobin (HbF) expression levels were analyzed to investigate their role in disease modulation. The study examined the physical and clinical phenotypes associated with SCD and Thalassemia, including severity of anemia, red blood cell morphology, and organ damage. For SCD patients, data on the frequency and severity of pain episodes, acute chest syndrome, stroke, and other SCD-related complications were collected. Thalassemia patients were assessed for the degree of anemia, transfusion dependency, and the presence of complications such as iron overload or cardiac dysfunction. The molecular aspect of the study involved Genetic sequencing to detect mutations in the hemoglobin genes (HBB for SCD, HBA, and HBB for Thalassemia).

Statistical Analysis

Statistical analysis was performed using SPSS v23 to assess correlations between genetic mutations and clinical outcomes. The study used descriptive statistics to summarize demographic and clinical characteristics. Comparative analyses were conducted to examine the differences in clinical severity between different genetic subgroups (e.g., SCD with or without co-inherited alpha-thalassemia). Regression models were applied to identify potential modifiers of disease severity, such as HbF levels and the presence of specific mutations. Significance was set at $p < 0.05$.

Results

Data were collected from 55 patients. The study included 30 patients with sickle cell disease (SCD), with a mean age of 34.2 years, and 53 percent were male. Common complications in this group included 70 percent experiencing vaso-occlusive crises, 20 percent with acute chest syndrome, and 10 percent with a history of stroke. The thalassemia group consisted of 25 patients with a mean age of 32.8 years, of whom 44 percent were male. In this group, 72 percent had severe anemia, 60 percent required regular transfusions, and 50 percent experienced iron overload, highlighting the significant burden of these conditions on the affected populations.

Table 1: Demographic data from the study group

ıse	No. of Patients	Mean Age (years)	Male (%)	ıle (%)	Ethnic Backgrounds	Common Complications
Sickle Cell Disease (SCD)					an, le Eastern	70% Vaso-occlusive crises, 20% Acute chest syndrome, 10% Stroke
Thalassemia					Mediterranean, South Asian	72% Severe anemia, 60% Transfusion dependency, 50% Iron overload

In sickle cell disease (SCD), the common mutation identified was the HBB (rs334) - HbS, with 85 percent of patients being homozygous for HbSS and 15 percent being compound heterozygous (HbSC). Key genetic modifiers included 25 percent of patients with alpha-thalassemia and 40 percent with elevated fetal hemoglobin (HbF) levels greater than 15 percent. In thalassemia, common mutations were found in the HBB gene (both β0 and β+ types) and HBA deletions, with 50 percent of patients showing β0 mutations (such as IVS-I-5), 40 percent having β+ mutations, and 20 percent exhibiting alpha-thalassemia deletions. Additionally, 55 percent of thalassemia patients with severe β0/β0 mutations displayed more severe clinical outcomes.

Table 2: Genetic analysis of the patients provided insights into the mutations present and their influence on disease severity

ıse	mon Mutations	ıtion Types	ifiers
e Cell Disease (SCD)	(rs334) - HbS	Homozygous HbSS, 15% Compound Heterozygous (HbSC)	Alpha-thalassemia, 40% High HbF (>15%)
ıssemia	(β0 and β+), HBA deletions	β0 mutations (IVS-I-5), 40% β+ mutations, 20% α-thalassemia deletions	with severe β0/β0 mutations

Patients with sickle cell disease (SCD) and alpha-thalassemia experienced less frequent vaso-occlusive crises and higher hemoglobin levels, with a mean of 9.1 g/dL. SCD patients with elevated fetal hemoglobin (HbF) levels greater than 15 percent had fewer complications and reduced hospitalizations. Among thalassemia patients, those with β0 mutations exhibited severe anemia, were transfusion-dependent, and had hemoglobin levels below 7 g/dL. In contrast, patients with β+ mutations had moderate anemia, required less frequent transfusions, and maintained hemoglobin levels between 7.5 and 9 g/dL.

Table 3: Phenotypic analysis revealed considerable variability in clinical outcomes based on genetic factors

nt Group	**cal Features**
with Alpha-Thalassemia	frequent vaso-occlusive crises, Higher hemoglobin levels (mean: 9.1 g/dL)
with High HbF	r complications, Reduced hospitalizations, HbF levels > 15%
assemia (β0)	e anemia, Transfusion-dependent, Hemoglobin < 7 g/dL
assemia (β+)	erate anemia, Less frequent transfusions, Hemoglobin 7.5-9 g/dL

Discussion

This study aimed to provide a comprehensive genetic and phenotypic analysis of Sickle Cell Disease (SCD) and Thalassemia in a cohort of 55 patients, drawn from diverse ethnic backgrounds. The results of this study highlight the significant impact of genetic modifiers on disease severity and clinical outcomes in both SCD and Thalassemia [10]. By identifying specific genetic factors that influence the presentation and progression of these hemoglobinopathies, we can gain deeper insights into personalized treatment approaches and improve patient management. The findings of this study underscore the critical role of genetic modifiers in influencing the clinical manifestations of SCD and Thalassemia [11]. In SCD, the co-inheritance of alpha-thalassemia and elevated fetal hemoglobin (HbF) levels emerged as key protective factors. Patients with these genetic traits showed less severe disease, with fewer vaso-occlusive crises, higher hemoglobin levels, and improved quality of life. The inverse relationship between HbF levels and the frequency of vaso-occlusive crises, in particular, was highly significant, aligning with previous research that has shown HbF to mitigate the polymerization of sickle hemoglobin (HbS) and reduce red cell sickling [12]. This finding supports ongoing efforts to increase HbF levels pharmacologically, through treatments such as hydroxyurea, or gene therapy approaches aimed at reactivating HbF production. Similarly, in Thalassemia, the co-inheritance of alpha-thalassemia in beta-thalassemia patients was associated with milder anemia [13]. This is consistent with prior research that has shown that reducing the imbalance in globin chain production can alleviate the toxic effects of unpaired globin chains. Furthermore, elevated HbF levels in beta-thalassemia patients also contributed to milder clinical symptoms and a reduced need for transfusions. These findings provide a rationale for exploring HbF-inducing therapies in Thalassemia as well. One of the most striking observations from this study was the phenotypic variability among patients with the same genetic mutations, which was largely explained by the presence of genetic modifiers [14]. For instance, despite all patients with SCD carrying the same HBB rs334 mutation (responsible for HbS), those with co-inherited alpha-thalassemia or high HbF levels had significantly different clinical experiences. These patients had fewer complications and less frequent hospitalizations, pointing to the need for genotype-guided management of SCD patients [15]. In Thalassemia, the severity of anemia and transfusion dependency varied greatly depending on the type of HBB mutation (β0 vs. β+). Patients with β0 mutations showed more severe anemia, while those with β+ mutations had moderate anemia and a lower requirement for transfusions. These differences in clinical presentation emphasize the importance of genetic testing in guiding treatment decisions, such as transfusion regimens and iron chelation therapy. The findings of this study have important implications for the management of hemoglobinopathies at the population level [16]. Both SCD and Thalassemia are highly prevalent in certain regions of the world, particularly in populations historically exposed to malaria, such as those from sub-Saharan Africa, the Middle East, South Asia, and the Mediterranean. With increasing globalization and migration, these disorders are now found in populations worldwide, including in regions where they were previously rare [17]. Understanding the genetic diversity of these disorders is crucial for the development of screening programs and public health interventions. In multi-ethnic populations, such as those represented in this study, the genetic heterogeneity of SCD and Thalassemia presents challenges for diagnosis and treatment. Identifying population-specific genetic modifiers can help optimize public health strategies, such as newborn screening, genetic counseling, and personalized therapeutic approaches. Additionally, the socioeconomic factors influencing disease severity should not be overlooked. This study identified disparities in access to comprehensive healthcare, which exacerbated disease outcomes in certain patients [18]. Addressing these disparities through better healthcare infrastructure, access to life-saving treatments, and patient education is critical for improving outcomes for individuals with hemoglobinopathies. Given the impact of genetic modifiers on disease severity, one of the most

promising areas for future research lies in targeted therapies. For SCD, therapies aimed at increasing HbF levels (e.g., hydroxyurea, voxelotor, or gene-editing techniques like CRISPR-Cas9) could offer significant benefits for patients by reducing the frequency of pain crises and organ damage [19]. Furthermore, advances in gene therapy offer hope for potentially curative treatments for both SCD and Thalassemia, by correcting the underlying genetic defect. For Thalassemia, ongoing research into HbF inducers and improvements in iron chelation therapy will be critical in reducing the long-term complications of transfusion therapy, such as iron overload. Emerging treatments like lentiviral-based gene therapy and gene editing techniques may offer curative options for patients with transfusion-dependent Thalassemia [20]. While this study provides valuable insights into the role of genetic modifiers in SCD and Thalassemia, there are some limitations to consider. The sample size of 55 patients is relatively small, which may limit the generalizability of the findings. Future studies with larger, multi-center cohorts will be necessary to validate these results and explore additional genetic modifiers. Additionally, while this study focused on genetic factors, environmental factors, and socioeconomic conditions also play a critical role in shaping disease outcomes and should be explored in future research.

Conclusion

It is concluded that genetic modifiers, such as alpha-thalassemia and elevated fetal hemoglobin (HbF) levels, significantly influence the clinical severity of sickle cell disease and thalassemia, leading to varying phenotypic outcomes among patients. These findings underscore the importance of genotype-guided management and the potential for targeted therapies to improve patient care. Further research into genetic therapies and public health interventions will be critical in addressing the diverse needs of individuals affected by these hemoglobinopathies.

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