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Distribution of Hemoglobinopathy Disorders in Saudi Arabia Based on Data from the Premarital Screening and Genetic Counseling Program, 2011-2015

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ABSTRACT

Distribution of Hemoglobinopathy Disorders in Saudi Arabia Based on Data from the Premarital Screening and Genetic Counseling Program, 2011-2015
By Eman Saud Alsaeed

PURPOSE: The prevalence rates of β –thalassemia (β-thal) and Sickle Cell Disease (SCD) in Saudi Arabia are considered one of the highest compared to surrounding countries in the Middle East (0.05% and 4.50% respectively). In 2004, Saudi Arabia introduced a mandatory premarital screening and genetic counseling program (PMSGC) as a preventive program for these genetic diseases. We analyzed data provided by this program from 2011-2015 to assess recent trends in β-thal and SCD and their geographic distribution in Saudi Arabia.

METHODS: Our secondary data analysis included 1,230,582 individuals. The de-identified database was obtained from the Department of Genetics of the Saudi Ministry of Health (MoH) which houses the ongoing premarital test program, and included all couples attending the program from February 2011 to December 2015. The status of β-thal and SCD was categorized as positive, negative, and trait forms.

RESULTS: During the 5-year study period, the overall prevalence rate per 1000 population for β-thal was 13.6 (12.9 for the trait and 0.7 for the disease). The prevalence rate for SCD was 49.6 (45.8 for the trait and 3.8 for the disease). Rates for β-thal were found to decrease from 24.2 in 2011, to 12 in 2015. However, SCD rates remained rather constant and ranged from 42.3 in 2011 to 49.8 in 2015. The highest rate for both β-thal and SCD was observed in the Eastern region and Jazan with different variation between disease and carrier statuses.

CONCLUSION: The overall rate for β-thal and SCD has shown a decreasing trend over the 5-year study period. This reflects major accomplishment of the premarital screening and genetic counseling program. We recommend further improvement in the preventive measures in the high-risk regions, and enhanced community awareness to provide the highest rate reduction for these disorders.

Keywords: Hemoglobinopathy, thalassemia, sickle cell disease

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Term Definitions

Alpha-thalassemia	α-thal
Beta-thalassemia	β-thal
Beta- thalassemia trait	β-thal trait
Beta-thalassemia major	β-thal major
Centers for Disease Control and Prevention	CDC
Complete blood counts	CBC
Confidence intervals	CI
High-performance liquid chromatography	HPLC
Identification	ID
Institutional Review Board	IRB
Kingdom of Saudi Arabia	KSA
Mean Corpuscular Hemoglobin	MCH
Mean Corpuscular Hemoglobin	MCH
premarital screening and genetic counseling programs	PMSGC
Sickle Cell Trait	SCT
Sickle cell disease	SCD
Virginia Sickle Cell Anemia Awareness Program	VaSCAP
World Health Organization	WHO

Chapter 1. Introduction

Overview on Hemoglobinopathy Disorders

Hemoglobinopathy, which are genetic disorders of hemoglobin, are the most common inherited diseases in humans (1). Global estimates indicate that approximately 300,000–400,000 infants are diagnosed yearly with hemoglobin disorders (2). In 2011, the World Health Organization (WHO) reported that about 5% of the world's population was carriers of hemoglobin disorders (3).

The prevalence of hemoglobinopathy is increasing globally, which represents a major public health problem (1, 4). Some forms of these disorders are considered serious autosomal recessive phenotype disorders, such as Beta-thalassemia (β-thal) major, a lethal genetic disorder (5). In developed countries, genetic diseases including hemoglobinopathy disorders consumed an 40% of the medical supplies to provide the care for these disorders (2). There has also been a significant increase in the mortality rate for hemoglobinopathy disorders over years.

Two hemoglobinopathy disorders, thalassemia disorder and sickle cell disease (SCD), have been the focus of significant attention due to increases in mortality and morbidity rates attributed to them (6). Yearly, approximately 240 million cases of heterozygous beta-thalassemia (β-thal) are diagnosed worldwide, mostly in the Mediterranean islands and in parts of Southeast Asia (6, 7). SCD incidence in newborns was estimated to be 300,000 in 2008 (7). SCD prevalence has significantly increased in most of the sub-Saharan Africa, the Mediterranean, and the Middle East (7).

Preventive screening programs have been adopted in several countries worldwide to

reduce the prevalence of β-thal and SCD (13). First, a premarital screening program was established in 1976 in Rome to determine the β-thal traits among students in a group of intermediate schools (14). This was followed in 1980 by the establishment of an SCD preventive program in Virginia (15). After this, many countries worldwide also adopted different types of preventive programs: premarital carrier screening, family-oriented approach to prevention, neonatal screening, antenatal screening for chromosome abnormalities and congenital malformations, and pre-implantation genetic diagnosis (12). Countries that successfully implemented these programs included Italy, Greece, Cyprus, France, Iran, Thailand, Australia, Singapore, Taiwan, Hong Kong, and Cuba (13). The goal of complete eradication for thal was met in Cyprus, Italy, and Greece (16).

In the Middle East, eight countries have established premarital screening and genetic counseling programs (PMSGC) that require that couples be tested before receiving a marriage license. Genetic counseling is provided to couples found to be at risk for hemoglobinopathy disorders (16).

Status of Hemoglobinopathy Disorders in Saudi Arabia

In 1963, Saudi Arabia discovered the presence of SCD, β-thalassemia, and other hemoglobinopathy disorders in its Eastern Region (8). After this, several epidemiological studies were done to identify the prevalence of hemoglobinopathy disorders (8). The prevalence rates for β-thal and SCD in Saudi Arabia are considered among the highest in the Middle East; rates in 2011 were 0.05% for β-thal and 4.5% for SCD (9). These high numbers are attributable to many factors but one of the strongest factors is the high rate of consanguineous marriage (10). Out of all marriages in Saudi Arabia, 34% occur between first-degree cousins (11, 12).

Saudi Arabia introduced a its own PMSGC in 2001 and made it mandatory in 2004 (17).

Along with this program, several supporting programs have also been developed, including educational initiatives and a neonatal screening program, to help reduce the rate of β-thal and SCD disease.

Study Significance

A handful of studies have been conducted in Saudi Arabia since pre-marital testing was made mandatory in 2004; they have shown a minor decline in the prevalence of SCD across Saudi Arabia, and mixed findings regarding the change in the thalassemia prevalence. A high amount of variability exists in the prevalence of β-thal and SCD between regions (9). In 2011, 6 years after the program was implemented, researchers found a marked decrease in the number of at-risk marriages and predicted a considerable reduction in the genetic disease burden in Saudi Arabia in the coming years (10). To our knowledge, no study has examined trends in prevalence rates of β-thal and SCD in Saudi Arabia since 2011.

A study done in 2015 determined the prevalence of SCD, SCT, and β-thal among patients attending the hospital emergency rooms. The prevalence of SCD was 96% among these patients, and 3.3% were SCT among the hospital attendees, with significant variations among the age groups(1). Overall, there is still a great need for reducing the burden of hemoglobinopathy disorders, specifically the β-thal and SCD in Saudi Arabia. The mandatory premarital testing program will contribute to this target. Ongoing monitoring of trends in hemoglobinopathy disorders over time is necessary to evaluate the success of the PMSGC program in achieving the targeted reduction, and eventually elimination, of disease burden.

To this end, we will conduct a study to assess recent trends in β-thal and SCD and their distribution by demographic characteristics and geographic regions in Saudi Arabia using data from the PMSGC program for the period of February 2011 to December 2015. We will also

examine the changes in current rates in comparison to previously reported estimates from 2004-2009. Our findings will help health officials monitor trends in hemoglobinopathy disorders over time. This data will inform an evaluation of the effectiveness of current prevention and control efforts and provide information useful for policymakers interested in reducing the prevalence of hemoglobinopathy disorders.

Research Questions

Our specific research questions are:

1. What is the time trend in the prevalence of β-thalassemia and SCD in Saudi Arabia for the period of 2011 to 2015?
2. Do prevalence rates for β-thalassemia and SCD vary by geographic regions in Saudi Arabia?

Chapter 2. Literature Review

This literature review will include studies concerning hemoglobinopathy disorders, more specifically β-Thalassemia (β-thal) and sickle cell disease (SCD), with different classifications of each disorder. Following that, we will discuss the global burden of hemoglobinopathies and the influential factors that have a direct impact on the incidence of hemoglobinopathy worldwide. After that, we will focus on studies on the increase in the frequency of these blood disorders in Arab countries and devote particular attention to Saudi Arabia. Finally, we will review the literature on preventive programs for hemoglobinopathy disorders, especially premarital testing programs like the one in Saudi Arabia.

Hemoglobinopathy Disorders

Hemoglobinopathy disorders occur due to genetic changes that cause defects in hemoglobin. More than 1,000 hemoglobin disorders have been identified and classified under two types of abnormalities. The first is a quantitative abnormality in the production of the hemoglobin subunit chains, alpha (α) and/ or beta (β), which generates thalassemia disorders (19). The second is a qualitative defect in the structure of hemoglobin subunits, which produces hemoglobin variants that give rise to sickle cell disease and other disorders. (19).

Thalassemia

Thalassemia is the result of genetic defects that affect the synthesis of alpha-hemoglobin and/ or beta-hemoglobin, resulting in their reduced or absent production. Deficiencies or absences in other globin subunits, delta (d)- or gamma (g)-globin, are also possible and can present in rare clinical manifestations (19).

Alpha-thalassemia (α-thal**)** This disorder is a commonly inherited heterogeneous disorder. Different forms of alpha-globulin subunit deletions in either one of the four genes encoding

alpha-globulin will present as distinct forms of this disease, all of which present in a milder form of thalassemia. α-thal usually presents as asymptomatic anemia with mild microcytosis and is occasionally misdiagnosed as iron deficiency anemia (20). If the deletion occurs in two alpha genes on the same chromosome, it will present as alpha zero thalassemia (19). Alpha zero mutations, generally cause pregnancy complications and result in stillbirth (2). If three alpha genes deleted, Hemoglobin H disease will be the outcome, and if four genes are absent, the baby may be born with a serious condition called hydrops fetalis and elevated Hemoglobin Barts (21).

Beta-thalassemia (β-thal) A remarkably high epidemiological frequency of β-thal exists in the Mediterranean area, the Middle East, and the Far East. Also, a relatively high incidence has also been detected in the African region (6). β- thal has three clinical classifications:

1. **Beta-Thalassemia minor or trait (β-thal trait)**: The heterozygous presence of beta globulin subunits and mild reduction in the quantity of this globulin will present in this form (22). The typical laboratory formula for the hemoglobin arrangement for this condition is 92%–95% HbA, 3.8% HbA2, and variable amounts of HbF amounting to 0.5%–4%. This profile will favor the hematological features of microcytosis, hypochromic red blood cells. This form of anemia has no obvious clinical manifestations (22). A small portion of individuals diagnosed with β-thal trait present with mild anemia symptoms such as a headache, lethargy, fatigue, dizziness, and exercise intolerance. Furthermore, this presentation may overlap with anemia due to iron deficiency (23). A review article on the topic suggests that β-thal trait may increase the risk of infectious diseases, but decreases the risk cardiac disease (22).
2. **Beta-Thalassemia intermedia (β-thal intermedia)**: In this homozygous deficiency of beta-globin, a broader presentation of symptoms can occur compared with β-thal trait and

β-thal major (2). While no obvious clinical manifestations occur in this disorder, patients may show symptoms later in life such as splenomegaly and mild anemia (6). In a severe form, medullary expansion with a facial deformity and osteoporosis due to erythroid hyperplasia can develop (22).

3. **Beta-Thalassemia major (β-thal major):** This is a severe, life-threatening, homozygous form of β-thal. Cooley's anemia associated with this form. The laboratory picture characterizing the hemoglobin is a decreased amount or the absence of hemoglobin A (0–30%), hemoglobin A2 range of 2% to 5%, and hemoglobin F range of 70% to 95% (6). β-thal intermedia and major types had been determined by hemoglobin levels: under 7 g/d is major, and over 7g/d is intermedia (22). There is a significant presentation of β-thal major: severe anemia, splenomegaly, and abnormal development. The patient requires recurrent blood transfusions along with the use of iron chelation with Desferrioxamine B to eradicate the outcomes of iron overload. Even with blood transfusions, patients will have a higher mortality rate than those who do not have the blood disorder, and their life expectancy is estimated to be only 30 years (5).

Sickle Cell Diseases

Sickle Cell Diseases are one of the most common qualitative mutation disorders for the globulin structure, along with glucose-6-phosphate dehydrogenase deficiency and Heinz body hemolytic anemia, which will not come into focus in this literature review (19). This mutation is a modification in the red blood cell from a donut-shape into a crescent-shape, which alters the smooth motion of the red blood cell through the blood vessels, and causes decreases in the blood flow to target organs (3).

Structural Variations of SCD

1. **Sickle cell trait (SCT):** The typical picture of the red blood cells of these patients is 40% HbS and 56%–58% HbA. Occasionally, there is no clinical presentation of this type, but often, the symptoms of anemia will present if severe hypoxia has occurred for the individual (2).
2. **Sickle cell anemia/disease (SCD):** This is the usual presentation of SCD; a complete malformation of the red blood cells occurs, and the shape of the red cell becomes a crescent—this mutation is called sickling. As a consequence of this abnormality, the permeability of the red blood cells and cellular dehydration will occur. In addition, the crescent shape of the red blood cells is rigid, and that increases blood viscosity and capillary flow obstruction, which results in hypoxia, tissue necrosis, and organ damage (11). General clinical manifestations will range from acute vaso-occlusive crisis to organ death; these clinical manifestations described in depth in a report from the CDC by Melissa, et al (17).
3. **Other mixed forms: Hemoglobin sickle cell disease, sickle b-thalassemia disease, and hemoglobin S with other hemoglobin variants:** These forms are rare. In hemoglobin S companied hemoglobin C, patients often present with vaso-occlusive manifestations similar to those with SCT, but the presence of hemoglobin C in the red blood cells greatly increases potassium efflux and red cell dehydration (19). Sickle β-thal disease and hemoglobin S with different hemoglobin types were often present as clinically asymptomatic (19).

Global burden of hemoglobinopathy disorders

In 2001, Weatherall and Clegg estimated the incidence and geographic distribution of

hemoglobinopathy disorders (3) (2). The authors found that different forms of hemoglobinopathy disorders vary in incidence by geographic location. The incidence of thalassemia disorders differed according to the type of thalassemia. The global average number of diagnosed cases of thalassemia disorders was estimated approximately 240 million cases of heterozygous β-thal, mostly in the Mediterranean islands and in parts of Southeast Asia (6, 7) The distribution of the sickle cell gene was found to be higher in sub-Saharan Africa, where it reached approximately 250,000-cases/year (4).

Factors influencing the increased frequency of hemoglobinopathy disorders

Factors related to the frequency of hemoglobinopathy disorders vary by region. In sub-Saharan and northern Africa, places with high rates of hemoglobinopathy disorders, malaria plays a significant role in increased frequency of blood disorders via natural selection. Any change in the morphology of red blood cells will create resistance to P. falciparum malaria. Therefore, those who have SCD will have less severe malaria, will have longer, healthier lives than those who do, and will be more likely to marry and have children. In doing so, they will produce more offspring who have or are carriers of SCD. In this way, the overall frequency can increase over time. As observed in a study in Africa, the distribution of malaria in different parts of the continent has a relationship to the distribution of hemoglobinopathy disorders. Malaria is more common in West and Central Africa, regions that also have a higher occurrence of sickle cell disease. This explanation may account for the high incidence of hemoglobinopathy disorders, specifically sickle cell disease, in Africa (4) (7). This association has been shown in Saudi Arabia as well. In a review article, El-Hazmi et al, indicate that malaria endemicity is relevant to the national history of blood genetic disorders in Saudi Arabia. A higher rate of blood disorders is found in malaria endemic regions and increases in travel spread the disorders more

widely around the kingdom (8). Another key factor, especially in parts of the Middle East, is consanguineous marriage, which increases the risk of autosomal recessive hemoglobin disorders (3)(3). This concept has been evaluated in many studies. A cross-sectional study conducted in Lahore from 2001-2005 aimed to determine the frequency of β-thal major in different ethnic groups, different Pakistani regions and castes, in addition to determining the effect of consanguinity on disease transmission (4). Almost 500 thalassemia couples that were referred for prenatal diagnosis from all over Pakistan were included in this study. Results showed that 56% of these couples were first cousins and 20% were relatives (4). In addition, trans-abdominal chorionic villus sampling was taken from all pregnant participants at 10-16 weeks of pregnancy. Of this group, 78.6% were found to have β-thal mutations (4).

Improvements in medical care and public health standards also impact the epidemiological distribution of morbidity and mortality for hemoglobinopathy disorders (3). Babies and children with these disorders are living longer now than they did before due to improvements in diagnosis and treatment. Thus, there are more adults living with hemoglobinopathy disorders(3).

Distribution of hemoglobinopathy disorders in the Eastern Mediterranean and Middle East countries

A review done by Hamamy and Alwan in 1994 focused on different hereditary disorders in the Eastern Mediterranean Region, highlighting the key blood disorders for countries such as Cyprus (25). These inherited disorders caused a significant proportion of physical and mental complications for those affected (12). β-thal trait status represented a higher percentage of the blood disorders in different regions of the Eastern Mediterranean. The proportion of β-thal trait in Libya was 11.2%, one of the highest in the region (25). In addition, a high percentage of carriers were also found in Cyprus, where it ranged from 15-17% (25, 26). A very successful pre-marital screening program introduced in these countries for β-thal, which helped reduce the

incidence of β-thal 23%to 6% within 15 years (25).

Another review article published in 2011 examined sickle cell disease in the Middle East and Arab countries and evaluated the occurrence, distribution, clinical features, management, and prevention as well as gene interactions of the HbS genes with other hemoglobin disorders. Focusing on the distribution of SCD, the study divided Middle Eastern countries into three different parts: those in Southwest Asia and the Arabian Peninsula, which includes Yemen, Saudi Arabia, Kuwait, Qatar, Bahrain, United Arab Emirates and Oman; the Northern region of the Arabian Peninsula that includes Palestine, Jordan, Syria, Lebanon and Iraq; and the Arab countries of North Africa which include Egypt, Libya, Tunis, Algeria and Morocco (27).These three different geographical distributions measured different levels of SCD distribution. This review indicated that Southwest Asia, Saudi Arabia, and Yemen have the highest proportion of hemoglobinopathy disorders. Yemen is similar to western Saudi Arabia in the prevalence of SCD patients. This study also demonstrated variation in SCD incidence within each country (27).

Hemoglobinopathy disorders in Saudi Arabia

Several studies addressed hemoglobinopathy disorders in Saudi Arabia, examining the most common hemoglobin inherited disorders, the prevalence of these disorders, the regional distribution, and the methods used to prevent these disorders.

In a 1993 study, Saudi Arabia, Africa and India were found to have the highest prevalence rates of hemoglobinopathy disorders (5). The two most common hemoglobinopathy disorders in Saudi Arabia are β-thal and SCD. The presence of hemoglobin S occasionally coexists with other abnormal hemoglobin, thalassemia, and glucose-6-phosphate dehydrogenase deficiency (6). A significant proportion of those with hemoglobin S (20%) were found to be living in the Qatif Oasis of eastern Saudi Arabia (5).

A retrospective analytical study examined regional differences for β-thal and SCD and at-risk marriages for these conditions using national data from 2004-2009 (7). This study focused on 0.6% of the Saudi population by examining data obtained from the premarital screening and genetic counseling program. The prevalence of β-thal in couples was 18.5 per 1000 examined persons; 18 per 1000 were carriers and 0.5 per 1000 were cases. In addition, the prevalence of SCD was 45.1 per 1000 examined persons; 42.4 per 1000 were carriers and 2.7 per 1000 were cases (7).

A retrospective chart review of hemoglobinopathy patients was conducted from 2011 to 2013 in King Abdul-Aziz Medical City in Riyadh. The study included of 3,332 patients who were admitted for anemia treatment or had hemoglobinopathy diagnoses and determined the occurrence of β-thal, SCT, and SCD (1). Among those patients different age groups and both genders that had a positive diagnosis of SCD were included. Almost Ninety seven percent of the participants were found to have SCD, and an approximately three percent had SCT, and none had β-thal. Distributions of these conditions differed by age: SCD was diagnosed in 48.5% of children (1). These percentages make SCD the priority among the hemoglobinopathy disorders in Saudi Arabia.

A review article on the epidemiology of SCD in Saudi Arabia pointed out that SCD is a relatively common genetic disorder with a prevalence that varies significantly by area. In 2011, SCD carriers made up 2% to 27% of the population, and up to 1.4% had SCD (out of a total population of 23.98 million) (8). The review also indicated that sickle gene mutations can develop independently and spontaneously at least five times per mutation, so no single mutation can be attributed to SCD (8). A specific haplotype found in Saudi Arabia has its origin in the Indus Valley Harappa culture and is called the Arab-Indian haplotype. This haplotype is also

found in Bahrain, Kuwait, and Oman (8).

Regarding SCD mortality, data is lacking. A study in the Eastern Province of Saudi Arabia showed that 73% of those who died were under 30 years old and had severe SCD complications like acute chest syndrome followed by infection (8).

Prevention programs for hemoglobinopathy disorders

Early preventive programs for hemoglobinopathy disorders were started after a study published in 1980, when the first SCD screening program was studied in 1970, 1 out of 500 babies had sickle cell anemia in the United States. This prompted the introduction of mass screening programs using an expensive test (hemoglobin electrophoresis) to detect any carriers of the sickle trait. This program was called the Virginia Sickle Cell Anemia Awareness Program (VaSCAP). In addition, the program's application effects were studied in n a cohort study conducted from 1970 to 1982, which included 12,000 samples collected during premarital testing from couples that had the percentage to have a child with SCD or other serious hemoglobinopathy disorders. The study identified 74 sickle-trait couples that were at high risk of producing a child with SCD. In 1972, legislation was introduced in Virginia to encourage physicians to recommend voluntary premarital examinations for sickle testing (1). It was found that the desire for having children would be affected by knowing the risk of hemoglobinopathy disorders in the offspring. Overall, the participants had a positive attitude toward decreasing the incidence of SCD, which would become generalized to the larger public (9).

In regard to thalassemia disease, the first screening program was introduced in 1975. Silverstroni and others reviewed an epidemiologic study of 17,724 students in Latium, Rome. This study aimed to detect thalassemia and start prophylaxis for the disease complications, mainly Cooley's anemia. The study had two stages. In the first stage, blood samples were taken

from the entire intermediate school population (after consent had been received). Positive and suspected cases were asked to attend the health center for further investigation. In the second stage, thalassemia was confirmed in certain students and an examination of the hematological profile of each was made. Thalassemia was confirmed in 98% of those with carrier status. With the results obtained from this study, it was suggested that the screening be repeated yearly to cover a larger sample size. The study hypothesized that repeating this program would make a premarital program more acceptable (3). At the time of this study, genetic counseling and premarital testing were in the beginnings of their application.

Along with the above finding, there has been an increase in the population's awareness and a rise in education levels in most areas with a high prevalence of hemoglobinopathy disorders (such as the Mediterranean, including Sardinia, continental Italy, Greece, and Cyprus) (10). In addition, intensive education campaigns have been conducted for couples that have had a child with a hemoglobinopathy disorder in areas with a high prevalence (10).

Genetic counseling and molecular studies have also been introduced to the field of prevention, especially for the β-thal trait and disease patients. Most of these preventive programs have shown a level of success, resulting in substantial improvement in the parents' knowledge. In addition, a marked declined in the rate of those born with thalassemia disease had been observed (10).

In 2001, a review of inherited hemoglobin disorders was performed examining all the different forms of hemoglobinopathy disorders. In this study, the authors categorized countries in regard to the pattern of control and prevention of hemoglobinopathy disorders. Countries were grouped into three divisions. In Mediterranean countries, the combination of screening and antenatal diagnosis along with specialized clinics for specific blood disorders all helped in

reducing the prevalence of β-thal by 80% (11). In the second group, the richer industrialized countries had higher numbers of immigrants from high-frequency regions, which were reflected in the prevalence of hemoglobinopathy disorders. A low level of control and management was done, as there was difficulty reaching immigrant groups (11). Lastly, in developing countries, lack of access to services highly affected the mortality rate due to undiagnosed, untreated or under-treated disease (11).

The WHO has increased its own efforts to fight hemoglobinopathy disorders. WHO to determine SCD and β-thal has adopted two assemblies. In 2006, the resolution on SCD from the 59th World Health Assembly and resolution on thalassemia from the 118th meeting were adopted by the governing bodies of WHO, in response to the increase in the prevalence of hemoglobinopathy disorders (12).

In Arab countries, where there is a high prevalence of hemoglobinopathy disorders, several preventive programs have been initiated. Examples of such programs include premarital carrier screening, family oriented approach to prevention, neonatal screening, antenatal screening for chromosome abnormalities and congenital malformations, and pre-implantation genetic diagnosis (13).

Saudi Arabia is one of the eight Middle Eastern countries (Turkey, Iran, Palestinian territories, Jordan, Bahrain, Iraqi Kurdistan, and United Arab Emirates) where pre-marital screening is mandatory (14).

The program was first introduced in 2003. All couples planning to get married must have blood work done before receiving their marriage certificate. The results of the blood work are directed to a special genetic counseling clinic; both partners are informed of the results and given the prospective prognosis for their children if they proceed to marry. The decision to marry is

then left to the couple, and the marriage certificate is given to them with the lab result confirmation (12).

Having a successful mandatory premarital testing program in Saudi Arabia will contribute to reducing the burden of hemoglobinopathy disorders, specifically β-thal and SCD. Ongoing monitoring of trends in hemoglobinopathy disorders over time is necessary to evaluate the success of the PMSGC program in achieving the targeted reduction, and eventually elimination, of disease burden.

Chapter 3. Manuscript

Distribution of Hemoglobinopathy Disorders in Saudi Arabia Based on Data from the Premarital Screening and Genetic Counseling Program, 2011-2015

By Eman Saud Alsaeed

PURPOSE: The prevalence rates of β –thalassemia (β-thal) and Sickle Cell Disease (SCD) in Saudi Arabia are considered one of the highest compared to surrounding countries in the Middle East (0.05% and 4.50% respectively). In 2004, Saudi Arabia introduced a mandatory premarital screening and genetic counseling program (PMSGC) as a preventive program for these genetic diseases. We analyzed data provided by this program from 2011-2015 to assess recent trends in β-thal and SCD and their geographic distribution in Saudi Arabia.

METHODS: Our secondary data analysis included 1,230,582 individuals. The de-identified database was obtained from the Department of Genetics of the Saudi Ministry of Health (MoH) which houses the ongoing premarital test program, and included all couples attending the program from February 2011 to December 2015. The status of β-thal and SCD was categorized as positive, negative, and trait forms.

RESULTS: During the 5-year study period, the overall prevalence rate per 1000 population for β-thal was 13.6 (12.9 for the trait and 0.7 for the disease). The prevalence rate for SCD was 49.6 (45.8 for the trait and 3.8 for the disease). Rates for β-thal were found to decrease from 24.2 in 2011, to 12 in 2015. However, SCD rates remained rather constant and ranged from 42.3 in 2011 to 49.8 in 2015. The highest rate for both β-thal and SCD was observed in the Eastern region and Jazan with different variation between disease and carrier statuses.

CONCLUSION: The overall rate for β-thal and SCD has shown a decreasing trend over the 5-year study period. This reflects major accomplishment of the premarital screening and genetic counseling program. We recommend further improvement in the preventive measures in the high-risk regions, and enhanced community awareness to provide the highest rate reduction for these disorders.

Keywords: Hemoglobinopathy, thalassemia, sickle cell disease

Introduction

Hemoglobinopathies, genetic disorders of hemoglobin, are the most common inherited disease in humans (15). Global estimates indicate that approximately 300,000–400,000 infants are diagnosed yearly with hemoglobin disorders (2)(11). Two hemoglobinopathy disorders, thalassemia and sickle cell disease (SCD), have been the focus of significant attention due to increases in mortality and morbidity rates attributed to them (10) (3).

In the Middle East, eight countries have established premarital screening and genetic counseling programs (PMSGC), which constitute a mandatory step prior to receiving a marriage license and offer genetic counseling to couples at risk for hemoglobinopathy disorders (14). Saudi Arabia introduced this program in 2001 and made it mandatory by 2004 (16). A handful of studies have been conducted since 2004; they have shown a minor decline in the prevalence of SCD, but there were inconsistent reports about the prevalence of thalassemia. After 6 years of launching program, researchers found a marked decrease in the number of at-risk marriages and predicted a considerable reduction in the genetic disease burden in Saudi Arabia in the coming years.

Overall, there is a great need for reducing the burden of hemoglobinopathy disorders, specifically the β-thal and SCD in Saudi Arabia. Having a successful mandatory premarital testing program will contribute to this target. Ongoing monitoring of trends in hemoglobinopathy disorders over time is necessary to evaluate the success of the PMSGC program in achieving the targeted reduction, and eventually elimination, of disease burden. In the light of this situation, we conducted a study to assess recent trends in β-thal and SCD and their distribution by demographic characteristics and geographic regions reflecting by the time in Saudi Arabia using data from the PMSGC program for the period of February 2011 to December 2015. Our findings

will help health officials monitor trends in hemoglobinopathy disorders over time. This data will eventually contribute to ascertaining the effectiveness of current prevention and control efforts and provide information useful for policymakers interested in reducing the prevalence of hemoglobinopathy disorders.

Methods

Population

Saudi Arabia, located in the Arabian Peninsula in the Middle East, east of the Red Sea and west of the Persian Gulf, had an estimated population of 30,770,375 with an annual growth rate of 2.55 and fertility rate of 2.75 in 2014 (17).

Saudi citizens make up 67.3% of the total population; 39.8% of the population are under the age of 15 years and 67.6% are from 15-64 years old (17). In addition, the population is estimated to grow to 39.8 million by 2025 according to United Nations projections (18). Saudi Arabia is divided into thirteen administrative regions: Al-Jouf, Asir, Baha, Eastern region, Hail, Jizan, Maddinah, Makkah, Najran, Northern Borders, Qasim, Riyadh, and Tabouk.

These regions are subdivided into twenty health regions according to Ministry of Health (17). The highest proportion of the population is found in Riyadh, Jeddah, and Eastern Regions

Premarital screening and genetic counseling program

Saudi Arabia launched its premarital screening and genetic counseling program in 2004. A royal decree issued in 2003 mandated premarital screening for the genetic diseases β-thal and SCD as a requirement to obtain marriage certifications (19). Designated health centers were established for the program and equipped with medical supplies, personnel, and laboratory services (2). There are currently 125 premarital health-screening centers across Saudi Arabia, all

of which report data to the Ministry of Health, Department of Genetic Diseases (20). The program offers free testing and counseling for couples looking to get their marriage certificate (3).

The PMSGC program not only identifies genetic blood diseases but also includes some infectious diseases such as hepatitis B, C and Human Immunodeficiency Virus, as well as radiological examinations (19). After these screenings, couples will be provided with medical consultation in order to explain their chances of transmitting these diseases to their partner or future children, helping them plan healthy family outcomes (19).

For each partner in the health care center setting, the assigned program staff collects basic demographic information along with a medical history and general examination (19). Hemoglobinopathy screening includes complete blood counts (CBC), peripheral blood film, reticulocyte count, high-performance liquid chromatography (HPLC) and sickling test for all the blood samples (in EDTA anticoagulant) (7). The test of HPLC is performed even if CBCs are normal. Several hemoglobinopathy disorders can be detected from this analysis such as β-thal, SCD, and different variations of hemoglobin-like HbC (4). β -thal trait diagnosis is considered if a person has Mean Corpuscular Volume (MCV) <80 fL and/or Mean Corpuscular Hemoglobin (MCH) <27 pg, and a hemoglobin A2 level >3.2% (21). For diagnosis of the sickle cell trait, the test must show the presence of HbS with positive sickling (7).

Iron studies and serum ferritin, and DNA analysis are not routinely done along with the diagnosis of a-thal due to their low clinical significance in most of the cases (22, 23).

Data Source

We performed a secondary data analysis using the database of the Genetic department in MoH's, which houses the premarital screening program. We obtained genetic screening data on

all 1,230,582 individuals seeking to obtain a marriage certificate between the periods of February 2011 to December 2015. In 2011, data entry was started in February to be in computerized form. Blood samples were taken, the results were shared with all examinees, and genetic counseling was given. The database included demographic information as well as genetic test results for SCD and β-thal allowing classification of disease status positive, negative, and trait (1).

Ethical Considerations

The dataset was obtained from the data source in de-identified form. No patient identifiers were included, and a unique ID was used for each case in the dataset. Following protocol was followed to protect the privacy and patient's rights: while obtaining the data, recoding was done for the patient IDs to prevent any possibility of linking the data back to the subjects based on the use of this ID. Furthermore, the collected data doesn't contain any of the identifiers listed by the IRB. The study team fulfilled administrative and ethical approval requirements. This study was granted review exemption status by the Institutional Review Board (IRB) of Emory University because. After evaluation of the aims and objectives for the study, they determined that this study does not require IRB review because it did not meet the definition of "research" with human subjects or "clinical investigation" as set forth in the IRB policies and procedures and federal rules.

Study Variables

We determined what variables to include data in this study based on the 2011 study of Memish and Saeedi(24). The dataset include the following variables: a unique identifier to differentiate each individual in the data set, city, age (years), gender (male/ female), doctor's notes, test results for β-thal major, β-thal trait, SCD, and SCT (Positive/ Negative), and year of testing.

Data Management

Data was received as separate files with multiple lines per person reflecting each disease result for each individual, one file containing the positive test results and eight files containing the negative test results in the raw datasets, which there were approximately 154,336 observations in the positive file, and almost 7,522,356 observations in the negative files. Data was received in Excel format and analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). All subsequent data management, handling and statistical analyses were done using a terminal-end connection to a High Performance Computing Cluster at Emory University for further analyzing the data and saving results.

The SAS dataset was thoroughly examined for integrity, inconsistencies, inaccuracies, and invalid entries and changes were made to correct data errors and recode variables as needed. "Region" was recoded from its original 20-level variable representing health districts to a 13-level variable representing administrative districts, because population counts (denominators) were only available for administrative regions in Saudi Arabia. Reclassifications in the variable "City" were made to be consistent with the changes made in "Region". Tests for disorders/diseases not relevant for our study such as α-thal were removed (7). Entries outside the research timeframe were removed. When a disease was labeled 'other', the test result and notes were examined for any relevant information that might be important for our study. For a group of subjects whose test result was entered as "see comment", notes were examined to identify information that may inform "disease status" assignment. A total of 4588 individual who were described as possibly having either α-thal trait, iron deficiency, or silent β-thal or undetermined, RBC count, Hb, MCV, MCH, Hb A2, RDW, and Hb F in the β-thal traits, undetermined β-thal

or can't excluded in doctor comments (22). From these results, confirmed cases of β-thal trait were 653 were included in the positive for β-thal trait.

All datasets were separately cleaned and transposed by unique identifiers to create a one-line per person data structure and subsequently merged using SAS PROC SQL. Variable names were altered to retain the original source of common variables to check for inconsistencies and ensure completeness.

Duplicate entries in the negative datasets were examined, and the latest entry per person was retained. Of 15,971 subjects from the negative data set, 770 had positive test results for β-thal, from which 768 matched the test result in the positive data set and 15,126 had β-thal trait, from which 15,124 matched the test result in the positive data set.

Of 59,326 subjects from the negative data set, 188 had positive test results for SCD, from which 90 matched the test result in the positive data set and 2,243 had carrier test results for SCD, from which 1,868 matched the test result in the positive data set.

Subjects with positive test results were deemed as positive even if they had a contrasting entry in the negative dataset.

All files were merged to create a final dataset that included 1,230,582 observations with the following set of variables for each observer: subject identifier, gender, age, city, disease results (β-thal major, β-thal trait, SCD, and SCT), doctor notes, and year of testing.

Statistical Analysis

An exploratory analysis of the data was done and summary statistics for all independent variables were derived. Continuous variables were summarized with descriptive statistics (N, mean, standard deviation, range). Categorical variables were summarized with frequency counts and percentages within each category or between levels of a category as appropriate.

We estimated the prevalence rate and 95% confidence interval (CI) of β-thal and SCD overall, by year from 2011-2015, and for each region. Prevalence rates were calculated separately for disease and carrier statuses within each condition and estimated as: the number of affected individuals divided by the number of tested individuals per 1,000(25). Statistical significance was determined at the 5% level using two-sided P values. Rates were compared using rate ratios and 95% confidence intervals (CIs) (25).

Results

Population characteristics

The overall study population of 1,230,582 individuals attended the premarital screening centers distributed across the 13 administrative regions in Saudi Arabia between February 2011 and December 2015. Approximately there were 49.7% male with average age 27.8 years and (SD 8.85 years) and 50.34% female with an average age 22.6 years (SD= 6.4). Furthermore, a higher proportion of program attendees was in the 18-25 years age group; represented as 46.2 % for men and 53.8 % for women (Table 1). The population distribution over the years was as follows: 78072 in 2011, 258581 in 2012, 268097 in 2013, 276236 in 2014, and 305871 in 2015 (Table 2).

Prevalence rate, time trends and distribution of β-thal

Overall for the 5-year study period, the estimated prevalence rate for β-thal obtained from this study was 13.6 per 1000; 12.9 per 1000 (15,351 individual) for the trait and 0.7 per 1000 (787 individual) for β-thal major. Among the total number of people examined, there were 1,174,181 individuals with negative result for β-thal. The prevalence rate for the β-thal trait decreased from 24.2 per 1000 in 2011 to 12 per 1000 in 2015 (Table 1). The prevalence rate for β-thal major varied from 1 in 2011 to 1.6 in 2015. Among the β-thal cases, different variations were observed in different regions. Among the total number of the β-thal, the highest rate was present in Jazan region: (32.1 per 1000 [95%; CI 30.8-33.4]) for β-thal trait and (0.6 per 1000 [95%; CI 0.5-0.8]) for β-thal major (Table 3, Figuer3). The second highest rate was reported in the Eastern region rate was (23.7 per 1000[95% CI; 23.1-24.4]) for β-thal trait and (0.4 per 1000 [95% CI; 0.3-0.5]) for β-thal major (Table 3, Figuer3). Other regions showed rates that varied from 3 to 15 per 1000 for both β-thal trait and disease status. While β-thal rates showed a fluctuating pattern across regions by year, there was a general trend of decreased rates from 2011 to 2015 across the different regions, except for the northern region (Figure 1).

Prevalence, time trends and distribution of SCD

A higher prevalence rate was observed for SCD than β-thal; the overall rate was 5 per 1000 (4.58 (N=56,292) for the trait and 0.38 (N=4,632) for disease. A total of 1,169,408 screened negative for SCD. The total number of sickle tests done from 2011 to 2015 were 1,230,332; with Makkah having the highest number, followed by the Eastern region and Riyadh. The highest rate of SCT was present in Jazan: (135.7 per 1000 [95% CI; 133.2-138.2]) (Table 3, Figure 3). The second highest rate was observed in the Eastern Region: (114.4 per 1000 [95%

CI; 113.0-115.8]) (Table 3, Figure 3). In Asir, Al-Baha, Makkah rates were ranging from 31-42 per 1000. The lowest rate for SCT was observed in Hail (2.0 per 1000 [95% CI; 1.6-2.5]) (Figure 3). SCD was most prevalent in the Eastern Region (9.8 per 1000 [95% CI; 9.4-10.2]), followed by Asir and Jazan regions with their rates ranging between 6.8 and 7 per 1000. The lowest rate was present in Hail (0.1 per 1000 [95% CI; 0.0-0.2]) (Figure 2). Within each region, rates of SCD were rather constant across the 5 years (Figure 2).

Discussion

Our study assessed the prevalence, 5-year time trends, and distribution of β-thal and SCD in Saudi Arabia using data from the Saudi premarital screening and genetic counseling program from February 2011 to February 2015. We observed a decreasing trend in the prevalence of β-thal across the years; however, the prevalence of SCD remained rather constant.

Since the launch of the program, only one study published in 2011 has analyzed trends from 2004-2009. There were no available studies for comparison of the rates of β-thal and SCD post-2009.

Thalassemia is the most common genetic disorder in the world; the highest percentage of hemoglobinopathy disorders in KSA has been attributed to α-thal (39). Up to 45% of the population in the Eastern region of KSA is heterozygous for α-thal (40). However, because no biochemical diagnostic test is available for detection of α-thal carriers, and red cell morphology may be quite reasonable in hemoglobin electrophoresis (40, 41), the premarital screening program does not include this condition and it was not included in our study. On the other hand, β-thalassemia disorder accounts for the other portion of overall thalassemia prevalence. In our study, we found that 1.4% of the study population had β-thal and were considered high-risk as defined by the PMSGC program. The majority of these cases were β-thal carriers and the

remaining had β-thal major. This result is similar to the one from a 2004-2006 study that analyzed the first three years of data from the screening program (16). Though the disease proportions were similar, the carrier numbers were distinct. Compared to the previous study which reported a rate of 3.2% for β-thal carrier status, our study observed a rate of 1.3% (16).

Studies from other Arab countries reveal that the frequency of β-thal trait general falls within the range of 2.0% –10.0% (22). Our study reported regional variations within Saudi Arabia in β-thal major. The region of highest prevalence is arranged geographically along a belt from east to west, starting from the Eastern region toward the Western region (Baha, Jazan, and Makkah) (7).

In our study, Sickle Cell Disease represented the highest prevalence between the two-hemoglobinopathy disorders. The overall prevalence was 5% and the majority of cases were SCT. The number of cases was constant over the course of the study period. The number of SCD and SCT cases per year was 138,145. The current prevalence increased slightly compared to that reflected in previously published studies among the same population; the SCD rate went from 3 per 1000 in the previous study to 4 per 1000 in ours, and the SCT rate went from 42 per 1000 in the previous study to 46 per 1000 in ours (7). This increase may be explained by an increase in the disease survival rate attributable to improvements in health care services and utilization, so that more individuals with SCT or SCD are reaching marriage age (16, 18).

Comparing the regional spread of SCD and β-thal, we found that the highest prevalence for both occurred in the Eastern region and Jazan region, which is similar to the distribution reported in the 2004 – 2011 PMSGC study.

An important risk factor affecting the occurrence of both disorders, β-thal and SCD, is consanguineous marriage. Consanguinity is variously distributed around regions in Saudi Arabia

(7). Health approaches need to take these regional variations into consideration when implementing health promotion and healthcare services.

Malaria endemicity is reported to correlate with SCD occurrence. Our study reflects this pattern; the regional prevalence of SCD is consistent with the regional distribution of malaria, which is also reported to be highest in Jazan. Having SCD is known to confer a survival advantage and protective effect an against malaria. Those with SCD in malaria-endemic areas have a prolonged life expectancy and better survival rate for this disorder (7, 26).

One of the overall targets of the PMSGC program is to reduce and even eliminate the presence of β-thal and SCD in newborns. However, achieving this target is dependent upon the presence of other preventive programs such as prenatal screening and abortion; however, the application of these programs is restricted by ethical and societal values (19, 22).

One strength of Saudi Arabia's PMSGC program is the fact that it was made mandatory for those seeking marriage certificates. This means that everyone will gain awareness of hemoglobinopathy diseases, which will increase the efficacy of this program and forward its mission of being a strong preventive program for SCD, thal, and other diseases (e.g., hepatitis and HIV). This program has huge breadth, involving 4% of the total population every year, and provides a valuable data collection and research opportunity. Besides determining the change in trends of these disorders, the data contributes to a more accurate assessment of the magnitude of hemoglobinopathy disorders in Saudi Arabia.

This study had some limitations. The study would have required a longer timeframe to observe the genetic consequences of the PMSGC program (27, 28). We did not have access to data on the outcomes of the genetic counseling offered to those at risk of transmitting blood disorders to their offspring, so we did not know which couples decided to marry, and we could

not factor this into our analysis of the PMSGC program (24). Another limitation concerns the sample. Though our sample size was large, it may not reflect population characteristics for the smaller regions because of the smaller denominators of those regions.

In conclusion, while a decreasing trend in the prevalence of β-thal was observed from 2011 to 2015, the prevalence of SCD remained rather constant. Ongoing monitoring of disease rates is crucial to inform improvement in the premarital screening program and establishment of additional prevention programs for hemoglobinopathies.

Chapter 4. Conclusion and Recommendations

Saudi Arabia has one of the highest rates of β-thal and SCD, and to address this has Saudi Arabia has implemented a prevention program involving premarital screening and genetic counseling. We analyzed data form this program from 2011 to 2015 and our results showed that the program is moving towards reaching its goals of lowering the prevalence of β-thal; however, rates for SCD remain rather stable. Even though the overall prevalence of β-thal has dropped, regional variations exist and rates are still especially high among certain regions like Eastern Region and Jazan.

Since starting this program, several studies have been done and recommended areas for program improvement, which the MoH has applied. The care provided to β-thal and SCD patients has changed over the years, and full government support would help in achieving the target level of healthcare needed for the entire Saudi population.

Our study extends previous studies by providing an update on the recent trend in the occurrence of β-thal and SCD over a 5-year period. Our study revealed a marked reduction in β-thal but a steady state in sickle cell disease. Our data will set the stage for continued monitoring

of hemoglobinopathy disorders and will facilitate an evaluation of the effectiveness of existing preventive programs in the future. Our data yields important information for action for decision makers that will enable them to make decisions about program and policy changes.

Recommendation

To improve the effectiveness of the PMSGC program and lower the prevalence of hemoglobinopathy disorders, we recommend the following:

- β-thal and SCD rates in newborns should be assessed as one component of evaluating the effectiveness of the premarital screening program.
- Health promotion and disease prevention efforts should be improved for those at higher risk of transmitting blood disorders. There should be a follow-up program post marriage to ensure a sufficient level of awareness. In addition, Health education should be focused on younger age groups to improve their attitudes towards and receptivity to genetic counseling.
- Higher risk regions should be targeted for the establishment of health campaigns that will increase the population's level of awareness about the disorders and how serious they are.
- The media should convey messages around the risks of consanguinity.
- More effective genetic counseling and psychological support should be provided before marriage to ensure that at-risk couples have a comprehensive awareness of risks before they reach a decision about getting married.

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Figures and Tables

Figure 1. Prevalnce Rates° of β-thalassemia among differnt regions during years, Kingdom of Saudi Arabia, 2011 – 2015*

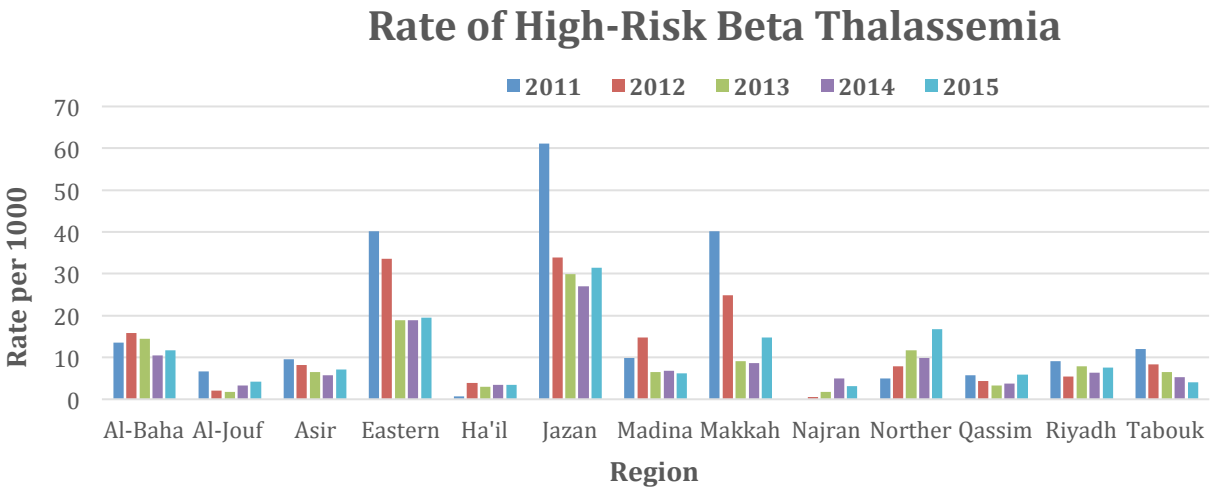

Figure 2. Incidence Rates° of High risk presention of Sikle cell disease among differnt regions Kingdom of Saudi Arabia, 2011 – 2015

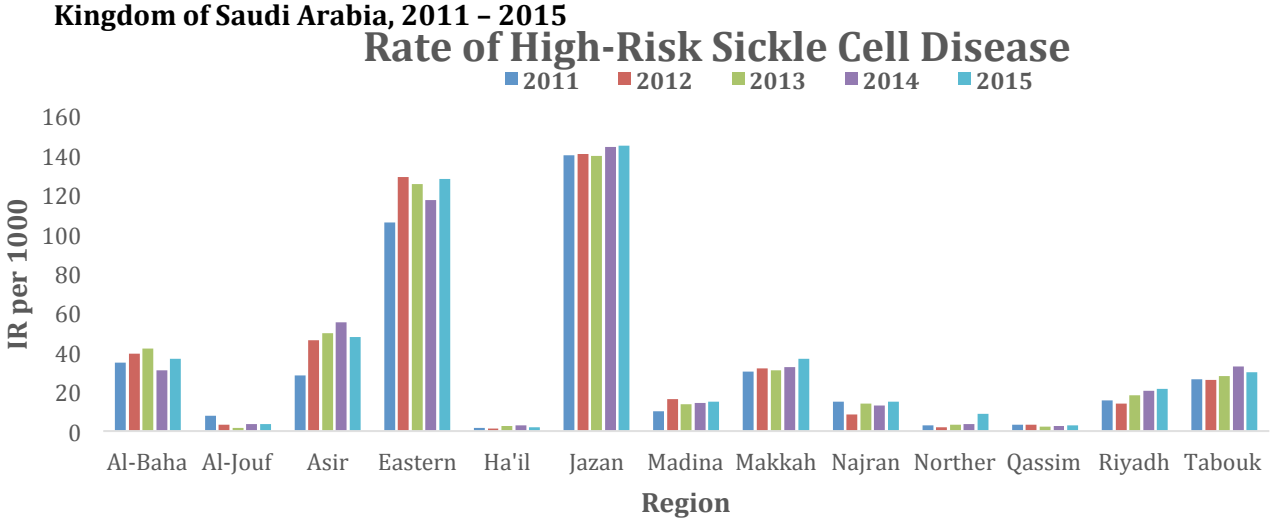

Figure 3. Prevalence Rates° for β-thalassemia and SCD carriers, by the highest rate region during years, Kingdom of Saudi Arabia, 2011 – 2015

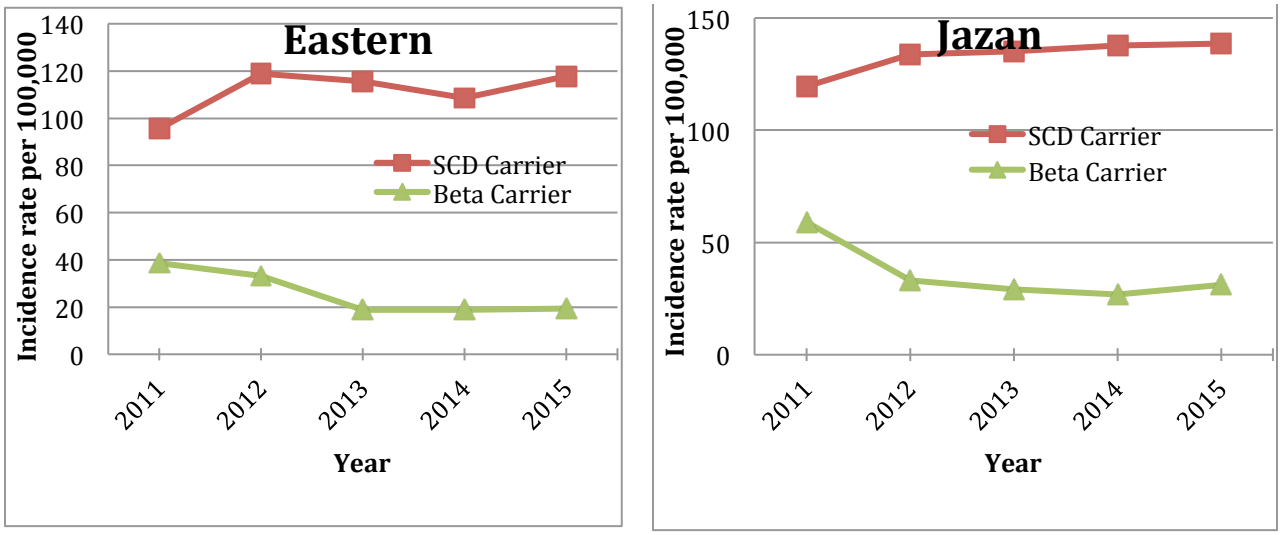

Table 1. Age distribution for men and women attending premarital screening in Saudi Arabia, 2011–2015

				Age groups (years)					
Gender	%	Age Range	Mean age ± SD	<18 (%)	18-25 (%)	26-35 (%)	36-45 (%)	46-55 (%)	>55 (%)
Males	49.7	17-105	27.8 ± 8.9	7.6	46.2*	64.4	66.1	83.8	24.1
Females	50.4	13-112	22.6 ± 6.4	92.4	53.8	35.7	34	16.2	5.3

*Statistically significant association

Table 2. Prevalence rates (PR per 1000) for β-thalassemia Trait and Sickle Cell Trait in Saudi Arabia form 2011 to 2015

	Population screened	**B-thalassemia Trait**			**Sickle Cell Trait**		
Year	1,230,582	Positive test	PR	Confidence Interval	Positive test	PR	Confidence Interval
2011	78072	1892	24.2*	23.2-25.3	3304	42.3	41.0-43.8
2012	258581	4557	17.7*	17.1-18.2	13055	50.5	49.7-51.3
2013	268097	2837	10.6*	10.2-11.0	13406	50.0	49.2-50.8
2014	276236	2666	9.7*	9.3-10.0	13490	48.9	48.0-49.7
2015	305871	3657	12.0*	11.6-12.4	15307	50.0	49.3-50.8

*p-value <0.0001 for comparisons of PRs for β-thalassemia carrier and Sickle Cell Trait for each year.

Table 3. Prevalence rate for β-thalassemia and Sickle Cell disorders by region, in Saudi Arabia, 2011–2015

		β-thalassemia				Sickle Cell Disorder			
Regions	Population	Trait	95%CI	Disease	95%CI	Carrier	95%CI	Disease	95%CI
Al-Baha	29161	13.2	11.9-14.6	0.3	0.2-0.6	34.9	32.8-37.0	2.8	2.2-3.5
Al-Jouf	29339	2.9	2.3-3.5	0.3	0.1-0.5	2.6	2.0-3.2	1	0.7-1.4
Asir	165316	6.7	6.3-7.1	0.2	0.1-0.2	42.5	41.5-43.4	7	6.6-7.4
Eastern Region	211727	23.7	23.1-24.4	0.4	0.3-0.5	114.4	113.0-115.8	9.8	9.4-10.2
Hail	38567	3.3	2.8-3.9	0.00	0.0-0.1	2.0	1.6-2.5	0.1	0.0-0.2
Jazan	72420	32.1	30.8-33.4	0.6	0.5-0.8	135.7	133.2-138.2	6.8	6.2-7.4
Makkah	238978	14.4	13.9-14.9	1.9	1.7-2.1	31.3	30.6-32	1.9	1.7-2.1
Maddinah	81286	8.1	7.55-8.79	0.2	0.1-0.3	13.7	13-14.6	0.8	0.6-0.9
Najran	24836	2.4	1.9-3.1	0.00	0.0-0.2	12.6	11.3-14.1	0.3	0.2-0.6
North Border	22260	7.6	6.5-8.8	3.4	2.7-4.3	4.0	3.3-5	0.4	0.2-0.8
Qasim	70057	4.0	3.6-4.5	0.4	0.3-0.6	2.5	2.2-2.9	0.3	0.2-0.5
Riyadh	200652	7	6.6-7.4	0.2	0.2-0.3	18.1	17.6-18.7	1.1	0.9-1.2
Tabouk	45983	6.3	5.6-7.1	0.2	0.1-0.4	27.5	26.0-29.0	1.0	0.8-1.4

95%CI= 95% confident interval

* Significant presentation among the B- thalassemia and Sickle cell disease p-value=0.0001