Epidemiology of Hemoglobin D in the Saudi Population

Mansour S Aljabry MD*

ABSTRACT

Rationale: Hemoglobin acts as a main molecular vehicle for oxygen transport. Genetic mutations in the hemoglobin gene can lead to structural and functional anomalies. Hemoglobinopathies are the most common monogenic inherited disorders. In the Middle East, hemoglobinopathies are most prevalent in the Kingdom of Saudi Arabia (KSA); however, no previous study reported the frequency of hemoglobin D (Hb D) in the Saudi population.

Methods: The data was extracted from the 1,872,495 entries in the Saudi eHealth Analytics (Seha) Platform of the Saudi Ministry of Health from subjects who enrolled for pre-marital screening. Complete blood count, hemoglobin electrophoresis, a sickling test, peripheral blood smears, reticulocytes, and serological testing for HIV, Hep B, and C was performed. Confirmation of hemoglobin bands in electrophoresis was performed through High-Performance Liquid Chromatography or capillary electrophoresis, or both. MATLAB was used to run the basic statistical analysis.

Results: The cohort (n= 1,872,495) comprised 49.8% males and 50.2% females with a mean age of 28.4±8.0 years (95% CI: 13–44). The rare hemoglobin variants were detected in 1825 individuals. The Prevalence of Hb D was n=754, with the highest prevalence in Mecca (n=290, 260), followed by Riyadh (n= 126, 121). Hb D occurrence per 10,000 of the study population is the highest in the Tabuk region (6.43 / 10,000). The mean RBS count, the mean MCV, and the mean MCH were in the optimal range.

Conclusion: No previous regional study has determined the prevalence of the rare Hb D variant. Our study reports the geographical prevalence of Hb D in KSA's 13 regions. These differences can guide future policymaking and practices.

Keywords: Hemoglobinopathies, Saudi Arabia, Hemoglobin D, Marital, Prescreening

INTRODUCTION

Defective hemoglobin synthesis, due to qualitative or quantitative anomalies, results in inheritable genetic disorders known as hemoglobinopathies, affecting 7% of the human population globally[1]. Severe hemoglobinopathies affect 300,000–400,000 newborns every year[2]. Hemoglobinopathies were originally found in the Middle East, Asia, and Africa. Kingdom of Saudi Arabia (KSA) has the highest incidence of hemoglobinopathies in the middle east[3].

Hemoglobin comprises two subunits, heme and globin. Globin gene mutations affect 7% of the human population globally[4]. Globin mutations are divided into two categories, mutations that affect the production of globin subunit and result in thalassemia and those which lead to abnormal production of globin proteins, also known as hemoglobin variants. The mutations leading to Hb variants are mainly missense mutations leading to more than 1000 hemoglobin variants. Hemoglobin A (dominant variant in adults), Hemoglobin A2, and Hemoglobin F (dominant variant in infants) are normal hemoglobin variants that are naturally occurring in healthy individuals[5]. However, a few variants, such as Hb C, Hb S, and Hb E, lead to negative clinical outcomes[6]. Besides these medically significant variants, hemoglobin D (Hb D) is the fourth most frequently occurring Hb variant, first described by Itano in 1951. Hb D is a group of at least 16 β-chain hemoglobin variants. The Hb D variant is characterized by glutamine at 121 position instead of glutamic acid in Hb A[7].

Hb D refers to a number of hemoglobin variant subsets, the most popular of which are D-Los Angeles, D-Punjab, and D-Ibadan[8]. These are hereditary variations of adult Hb A and have similar clinical effects. People of Asian Indian descent have the highest frequency of Hb D genes. But they can also be present in persons of European ancestry, particularly British and Irish[9].The four most typical hemoglobin patterns involving Hb D are precisely described here. The Hb D trait results from inheriting the gene for Hb D from one parent and a while Hb A gene from the other parent. The Hb D trait does not usually cause symptomatic health problems[10].Two screening specimens are tested for infants, and parents are advised for hemoglobin screening tests to determine the probability of having subsequent children with Hb sickle D (Hb SD) disease, an autosomal recessive condition. When both parents pass on the Hb D gene, the outcome is homozygous Hb D. In the first few months of life, moderate hemolytic anemia appears when fetal hemoglobin levels fall and Hb D levels rise, leading to decreased osmotic fragility[11]. When the gene for Hb D is inherited from one parent and the gene for Hb S (commonly known as a sickle cell) from the other parent, it results in compound heterozygotes with Hb SD illness. Early in life, fetal hemoglobin levels drop, and Hb S and D levels rise, leading to mild to severe hemolytic anemia. The phenotype is FDD in infants which changes to DD in adults.

Although a form of sickle cell disease, most individuals with Hb SD have fewer health problems and spleen involvement cases than the

* Associate Professor & Consultant Hematologist
Hematology Unit- Pathology department –
College of Medicine -King Saud University
Kingdom of Saudi Arabia.
E-mail: M.ALJABRY4210@Gmail.com; Maljabry@ksu.edu.sa

other more common forms. Hb D β-Thalassemia results due to co-inheritance of the gene for Hb D and β- thalassemia, which results in clinical manifestations ranging from mild to moderate. Splenomegaly and other clinical complications, such as iron deficiency, can occur[12].

KSA is thought to have one of the highest rates of Hb diseases among Middle Eastern nations, with sickle cell disease and thalassemia; being most prevalent and often reported in the eastern and southern parts of the country[13]. The Saudi Premarital Screening Program database indicated that 3.22% are carriers and 0.07% suffer from thalassemia[14]. The KSA government launched the PMSGC program in 2004, intending to enhance the quality of life, lower the prevalence of these diseases, and even eradicate Hb abnormalities from the Arabian Peninsula because hemoglobinopathies are amongst the most deadly and expensive conditions[15].Genetic disorders leading to Hb abnormalities are the most common monogenic diseases worldwide. Despite critical measures and discrete screening methods, hundreds of children with serious Hb disorders are born yearly. While estimating prevalence is paramount, research regarding hemoglobin variants is vital for two reasons. First, relating Hb gene mutations to hemolysis, cyanosis, and erythrocytosis in healthy people gives patients peace of mind and limits the need for additional diagnostic tests, saving money and reducing risk. Second, studying the structural, biochemical, and clinical implications of Hb variations has led to significant advancements in understanding how red blood cells work, given broad paradigms for protein biology. Despite critical measures taken by the KSA government, such as mandatory premarital screening, new cases are still emerging. A few case series have recently been published reporting on a rare hemoglobin variant, Hb D, in Saudi families[16,17]. Large-scale prevalence studies are necessary to estimate the total number of people suffering from hemoglobinopathies and establish guidelines for the proper management of patients and the eradication of these disorders in the near future. The current study aimed to determine the prevalence of Hb D in KSA for the first time, which will pave the path for future studies regarding hemoglobinopathies.

MATERIALS AND METHODS

Study Design: All individuals who applied for a premarital screening program and genetic counseling between 2009–2018 were included in the data collection process after receiving Institutional Review Board (IRB) approval for the current retrospective study. Seha Platform is the central database for the entry of data by laboratory supervisors, followed by results approval by the assigned physician. The Seha platform serves as an electronic repository containing all the necessary data points for those applying for premarital certificates, including patient demographics, laboratory findings, and the outcomes of the final pairing of couples. The reported data were pulled from the Seha platform from 13 designated regions of KSA.

The premarital screening program and genetic counseling (PMS&GC): The program infrastructure consists of 204 genetic counseling clinics, 172 linked laboratories, and 352 healthcare-receiving clinics (270 governmental and 82 commercial clinics) dispersed among the 13 administrative districts of KSA. Prior to receiving their marriage license, couples are required by PMS&GC policies to apply for pre-marriage screening at the closest pre-marriage center. The team at the PMS&GC acquired the fundamental demographic information, provided the essential health information, and took blood samples (in EDTA anticoagulant) for complete blood count, hemoglobin electrophoresis, a sickling test, peripheral blood smears, reticulocytes and serological samples for HIV, Hep B and C. High-Performance Liquid Chromatography (HPLC) or capillary electrophoresis, or both were used for confirmation of abnormal bands in hemoglobin electrophoresis samples. The result interpretation was performed according to standard lab protocols by physicians. Issuing of the final results and, if required, counseling sessions are done according to the program guidelines.

Statistical Analysis: Descriptive statistics were generated for continuous data, including minimum and maximum values, means, standard deviations (SDs), and frequencies for categorical variables. The categorical variables were tabulated with the frequency of occurrence. MATLAB v.2023a (Mathworks Inc, Natick, MA, USA) was used to merge data from different data sources of the Seha platform and run the basic statistical analysis.

RESULTS

The data were extracted from the Seha platform of 1,872,495 entries, with 49.8% male and 50.2% females with a mean age of 28.4±8.0 (95% CI: 13–44). Hemoglobin D variants were detected in 754 applicants (0.04%) of the total cohort.

Table 1 presents the total number of cases with the Hb D variant and its hematological characteristics. Calculation of homozygous DD, CC, or heterozygous SD trait is hemoglobin % dependent, where >50 indicates homozygous disease and <50 indicates trait. The total number of Hb D cases in our cohort was 754, where 17 were homozygous Hb DD, while nine were combined Hb SD disease. The rest of the cases were hemoglobin traits. Hb D cases had a mean hemoglobin% of 34.5 ±12.3 (males 34.3±13.6, females 34.7±11.1) and a hemoglobin level of 14.1 ±2.1 g/L. The mean RBS count was 5.3 ±0.6 $x10^{12}$/L, the mean MCV was 79.0 ±8.6 fL, mean MCH was 26.6 ±4.7 pg. All values were within the normal range specified in the local reference hematological indices[18].

Figure 1 shows Hb D was highest in the Mecca region (n=290, 260, 24, respectively), followed by Riyadh (n= 126, 121, 17). Hb D occurrence per 10,000 of the study population is the highest in the Tabuk region (6.43 / 10,000).

Figure 2A shows Hb D distribution and Figure 2B: depicts the relative percentages of HB D over the regions of KSA

DISCUSSION:

The current study aimed to assess Hb D prevalence trends and offer an update on Hb diseases in KSA. This is the largest data about the prevalence of hemoglobin D in Saudi Arabia The current study's results indicate a regional difference in the prevalence of Hb D in KSA. There was a total of 754 cases of Hb D, and the prevalence of Hb D was highest in the Mecca region (n=290, 260, 24, respectively), followed by Riyadh (n= 126, 121, 17). Hb D occurrence per 10,000 of the study population is the highest in the Tabuk region (6.43 / 10,000). The current study didn't find any effect of Hb D on mean hemoglobin, RBC count, and MCV.

The regional differences in prevalence can guide the policymaking for managing and eradicating hemoglobinopathies. Ours is the first study reporting on the prevalence of HB D in various regions of KSA. A case report of a Saudi family with three unique mutations was incidentally discovered in a bone marrow transplantation workup, where the father was compound heterozygous for Hb D while the mother was a carrier for β-thalassemia. Three children in the family were transfusion-dependent β-thalassemia, and two were compound heterozygous for Hb D. The other two children had Hb D traits, while two had β-thalassemia traits[17]. In another case report, a Saudi male patient was diagnosed with Hb SD

Table 1: The mean values and standard deviation of red cell indices and Hb F for the Hb D variant

Hemoglobin variant	*N* (%)	Mean hemoglobin% ±SD	Mean RBC (x10^{12}/L)	Mean MCV (fL)	Mean MCH (pg)	Mean hemoglobin level (g/L)	Mean HbF %
Hb D	754 (41)	34.5±12.3	5.3±0.6	79.0±8.6	26.6±4.7	14.1±2.1	0.53

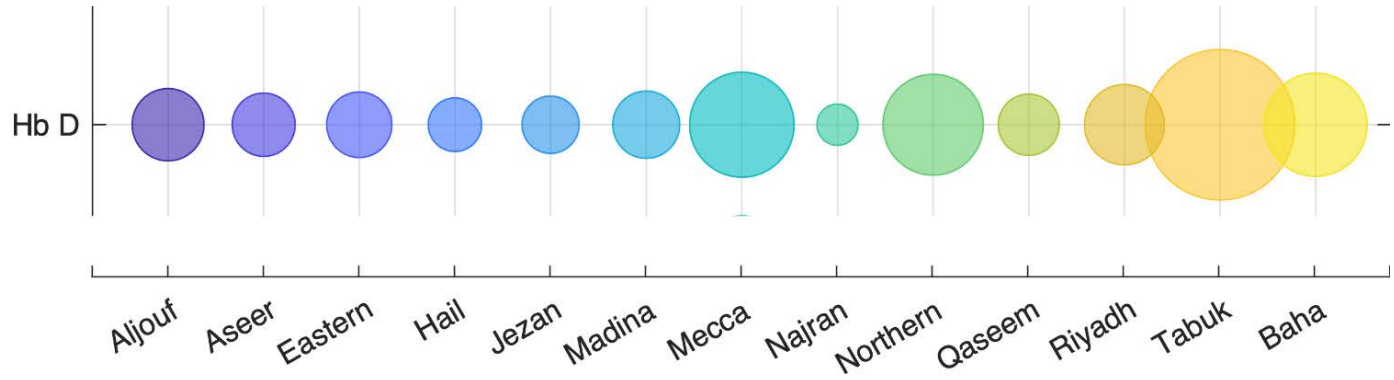

Figure 1: Bubble chart for Hb D in the study population, where the bubble size denotes cases per 10,000 in the 13 designated regions of the KSA

Figure 2A: Distribution of Hb D over the regions of KSA

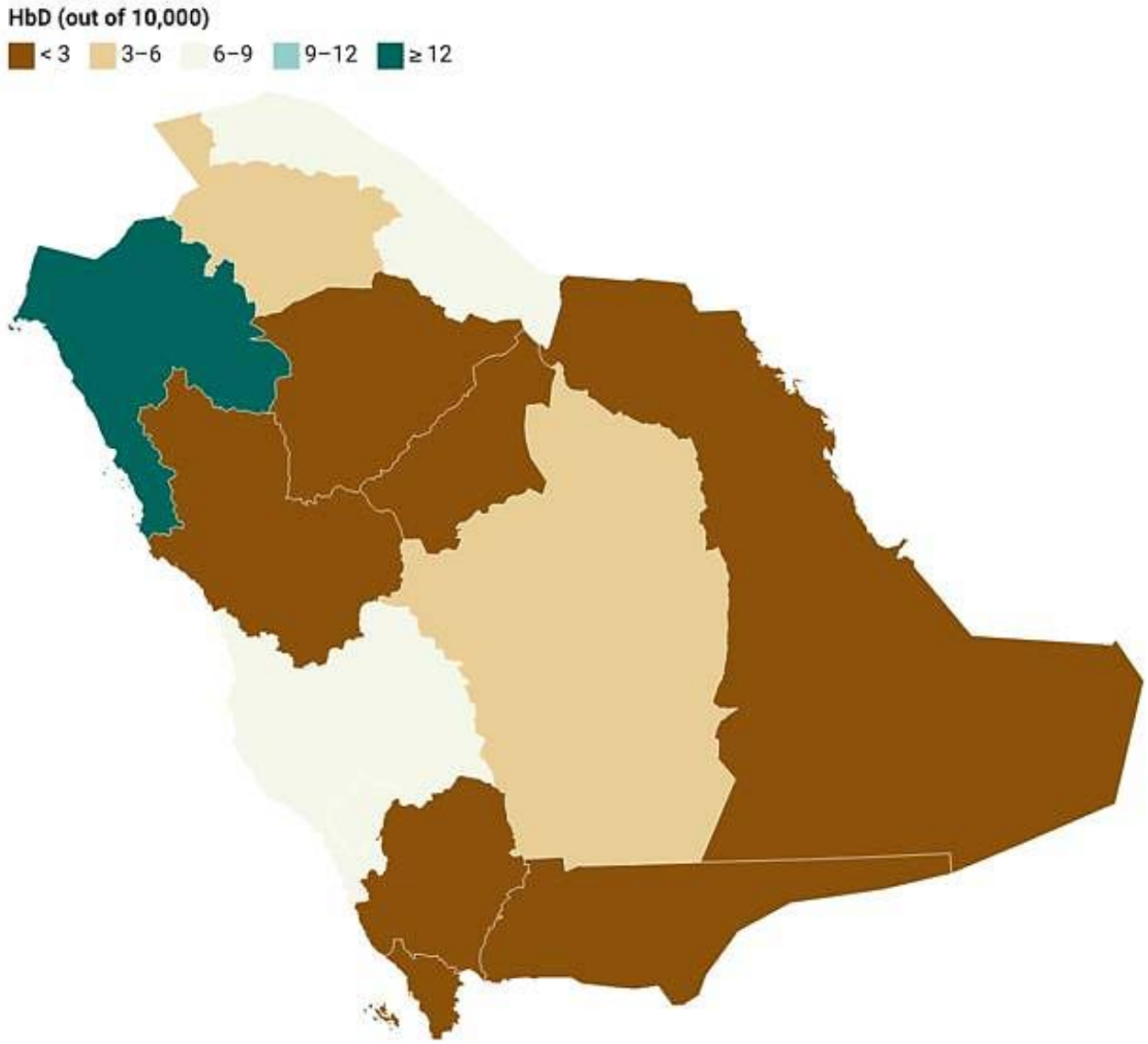

Figure 2 B: Relative percentages of Hb D over the regions of KSA

with a clinical presentation of renal complication and anemia[19].There was also a report of a rare combination of Hb D and alpha thalassemia in a Saudi family[16]. Prevalence studies can guide the workflow for such critical procedures, leading to better outcomes. These reports warrant careful molecular studies in premarital screening in order to manage such clinical cases.

The prevalence of hemoglobinopathies is different in ethnicities owing to its genetic component and positive genetic selection in areas with malaria endemic. It is more prevalent in North-Western India, where ~3.0% of the population is affected by this genetic alteration. However, the frequency drops by one-half in the western region of India by almost a half[20]. Hb D is also common in Pakistan (another region suffering from malaria)[21]. Thirty-three cases of Hb D have also been reported in Arab Emirates[22]. In our cohort, Hb D occurrence was the highest (6.43 / 10,000 per of the study population) in the northern Tabuk region, where most of the population comprises military personnel from all over the Kingdom, mostly from southern Jezan.

RBC index in Hb D patients is comparable to healthy controls as reported by Alaskar et al. RBC count in Hb D patients in our cohort was 5.3 ($\times 10^{12}$/L) while previously reported in male healthy control was 5.4 ($\times 10^{12}$/L), higher than the female group 4.5 ($\times 10^{12}$/L) in a previous study. MCH values in our Hb D cohort was 26.6 pg/cell while the mean MCH value in healthy men was 28.9 pg/cell and in women 27.69 pg/cell[18].

When inherited with Hb S, the different Hb D variants lead to significantly different clinical outcomes. Hb D Punjab results in clinical conditions such as sickle cell disease, while the other two variants, Hb D Ibadan and Hb D Iran, result in benign conditions such as Sickle cell trait[7]. The limitation of our study is the categorization of Hb D to Hb D Punjab or Iran.

Hb D-Punjab can be inherited as a homozygous illness, a heterozygous phenotype, or in combination with other Hb variations. Homozygous and heterozygous Hb D cases may be asymptomatic or have moderate microcytic hypochromic anemia. A combination of the Hb D trait with the Hb S trait or β-thalassemia trait, on the other hand, might result in symptomatic people. Hb SD leads to mild to moderate clinical symptoms owing to the polymerization of Hb S, which is stimulated by glutamine at 121 positions in Hb D (23). In the current study,

we reported 17 cases of Homozygous Hb DD disease that rarely occurs. The clinical presentation of Hb DD is characterized by mild microcytic hypochromic anemia and/or mild splenomegaly. It must be differentiated from Hb D β thalassemia as it presents with moderate chronic hemolytic anemia. Compound heterozygosity for Hb D and β thalassemia produce a mild thalassemia condition. Hb D-Punjab is common in Turkey, Belgium, Australia, and Italy. Hb D-Punjab is the second most common hemoglobin variant in Turkey. However, in south-eastern Turkey, Hb D is the most common variant affecting 0.2% population. 57.9% of abnormal hemoglobin observed in Denizil province of Turkey are Hb D-Punjab. Surprisingly, a similar frequency has been reported in Xinjiang province, northwestern China, where 55.6% of variant hemoglobin is Hb D-Punjab[9].The high prevalence of Hb D Punjab intrigued us to propose the high probability that the same haplotype can be found in Saudi nationals in future screening.

Our results indicate 7 cases of Hb SD. Clinical outcomes of Hb SD-Punjab patients reported recently are very similar to the Hb SS genotype. The patients were reported with vaso-occlusion episodes and/or required blood transfusions. The authors observed greater vulnerability for red cell lysis in Hb SD-Punjab patients than with the Hb SS genotype[9]. Clinical severity in Hb SD individuals is since Hb D favors the polymerization of Hb S. The occurrence of this phenotype in the Saudi population in the current study emphasizes the specific guidelines for managing this particular condition, just like sickle cell anemia.

The main reason for the prevalence of such cases is probably the high prevalence of consanguineous marriages (56%) in KSA. Royal decree made premarital screening for thalassemia and sickle cell anemia mandatory in KSA in 2004. If a prospective husband and wife are found to be carriers of thalassemia or sickle cell disease, or if any of them is homozygote for the illness, they are advised to consult the counseling centers and not to marry. KSA has implemented a countrywide program for newborn screening for hemoglobinopathies. In 2008, a scientific advisory group for Hereditary Blood Disease Centers (HBDC) was constituted in each area of KSA. In recent years, these interventions have significantly reduced the incidence of hemoglobinopathies in KSA. Typically, genetic screening programs focus on previously reported cases; nevertheless, carrier discovery is critical for disease prevention and ensuring the progeny's health. Being autosomal recessive conditions, the risk for both genders is similar. More research is indispensable to determine these variations' molecular and clinical significance concerning the unique genomic architecture.

CONCLUSION

The significance of the current paper is two-pronged: it's the largest data ever published reporting Hb D prevalence in KSA, and we also reported on combined Hb SD prevalence which is a clinically significant sickling disorder. More than a thousand hemoglobin variants occur naturally; only a few have clinical implications. Hb D is the fourth most common hemoglobin variant worldwide, affecting multiple countries like India, Pakistan, Turkey, Italy, and Iran. KSA is known for its high prevalence rate of hemoglobinopathies; however, none of the previous studies reported the prevalence of the Hb D variant in the country. For the first time, the current study reported the prevalence of Hb D in the 13 designated regions of KSA, with most cases in Mecca. The prevalence of rare variants like Hb D emphasizes the need for further longitudinal studies on these variants' biochemical and molecular attributes and their impact on quality of life. The results of the current study also reported rare homozygous cases, emphasizing the need for updates in clinical guidelines regarding these rare conditions. Future research must also elucidate in-depth genetic haplotypes of Hb and other rare hemoglobin variants prevalent in KSA. Lastly, there is a dire need to effectively enforce pre-marital screening and counseling to prevent further cases of such autosomal recessive disorders.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

Potential Conflicts of Interest: None

Competing Interest: None

Acceptance Date: 07-09-2023

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