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Distribution of Hemoglobinopathies among Premarital Couples in Al Majmaah, Saudi Arabia

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ABSTRACT

Hemoglobinopathies are inherited diseases of hemoglobin synthesis arising from mutations and/or deletions of one or more of the globin genes resulting in the production of abnormal hemoglobin molecules and subsequently reduced synthesis of normal alpha and beta globin chains in Hemoglobin molecules. In this study, we attempted to assess the distribution of hemoglobinopathies in Saudi Premarital couples planning to marry and applying for a marriage license in Al Majmaah region and attending the premarital screening center of King Khaled General Hospital (KKGH), Al Majmaah, Saudi Arabia. In total, 4009 cases were screened for hemoglobinopathies by using the Bio-rad Variant II cation exchange-high-performance liquid chromatography (CE-HPLC) system. The total number of abnormal hemoglobin fractions on cation exchange-HPLC (CEHPLC) was 127 cases. Sickle cell trait was the predominant genetic hemoglobin disorder accounting for 66 of the total cases. This was followed by the Beta-thalassemia trait in 50 cases, sickle-cell disease in 9 cases, HgbE disease in 1 case, and HgbD trait in 1 case. The outcome of this study indicated that the Saudi population in this area is at low risk for hemoglobin disorders. sickle cell trait and β-thalassemia trait were the most common Hb disorders in Al Majmaah, Saudi Arabia.

Key words: *Cation exchange-HPLC, Hemoglobinopathy, Hemoglobin disorders, Sickle cell disease, Thalassemia*

INTRODUCTION

Hemoglobinopathies are genetic (inherited) disorders of hemoglobin [1-11], the oxygen-carrying protein of the red blood cells. Normal adult hemoglobin contains four globin polypeptide chains (α_1, α_2, β_1, β_2), each with its hem molecule. These chains undergo conformational change and move concerning each other when binding O_2 and CO_2. 2,3 diphosphoglycerate (2,3-DPG) binds between the β chains to reduce affinity for O_2 and allow O_2 release to the tissues.

The globin genes can be found on chromosomes 11 (E, Y, B) and 16 (5, 0), respectively. The production of the y and B globin genes is regulated by a 5' locus control region (LCR). Prenatal and postnatal life involves the transcription of various genes, and the chains are separately synthesized before combining to create the various hemoglobins. At location 136, the y genes vary in that they can either generate a glutamic acid (Gy) or an alanine (Ay) residue. Hemopoiesis takes place in the yolk sac, liver, and spleen during pregnancy, but it is only allowed to occur in the marrow after birth.

Mutations and deletions in genes that encode these polypeptide chains cause one of the many hemoglobinopathies. Hemoglobinopathies are divided into those in which the gene abnormality results in a qualitative change in

hemoglobin molecule and those in which the change is quantitative. Sickle cell anemia and thalassemias are examples [1-11]. Hemoglobin *disorders* such as *β-thalassemia* and *Sickle cell disease* (*SCD*) *affect* millions of individuals worldwide [9]. β –thalassemia (β-thal) and SCD in Saudi Arabia were estimated to affect 0.05% and 4.50% of the population, respectively [10].

In 2003, the government of Saudi Arabia decided to implement a mandatory premarital screening program to decrease the incidence of the two most common hemoglobinopathies in Saudi Arabia, sickle cell disease and thalassemia. In this study, we attempt to assess the distribution of hemoglobinopathies among Saudi Pre-marital couples planning to marry and applying for a marriage license in the Al Majmaah region.

MATERIALS AND METHODS

Specimen collection and handling

The whole blood specimens were collected in a vacuum collection tube containing EDTA from King Khalid Hospital in Al-Majmaah in Riyadh province. Whole blood specimens are stable for seven days when stored at 2-8 °C or 48 hours at ambient temperature (22-24 °C). Samples were processed on VARIANT II b-thalassemia Short Program - Bio-Rad to detect any hemoglobinopathies for regular patients or individuals coming for premarital screening tests over three years from 2019 to 2021. A written informed consent was read and signed by all the participants in the study. All research procedures have been approved by the Ethical Committee, King Fahad Medical City, Ministry of Health, Kingdom of Saudi Arabia, approval number: IRB Log. Number: 19-644E. All procedures performed in this study were in accordance with the ethical standards as laid down in the Declaration of Helsinki as revised in 2013.

Specimen preparation

It wasn't necessary to prepare the sample. The sample containers are positioned on the conveyor track of the VARIANT I Sampling Station and inserted into the VARIANT I sample receptacles. Use specialized rack plugs for tubes that are 12, 13, and 14, and specialized adapters for infant tubes that are 10 millimeters. The sample was prediluted 1:200 before analysis. If it was in an unusually shaped or sized tube or if its height in the tube was less than 25 millimeters, the material was completely mixed before pipetting by carefully inverting the tube. Pipette 1.0 mL of Wash/Diluent Solution into a labeled 1.5 mL container, then add 5 uL of the whole blood sample to diluent the sample. After fully blending, seal the sample container with a cap. A microvial adapter was used for prediluted samples.

Reagents preparation

Elution buffers and wash/diluent solution

No preparation was required for buffers and wash/diluent solution. Each bottle of elution buffers1&2 contained 1900 and 1800 mL of sodium phosphate buffer respectively, while wash/diluent solutions contained 1800 mL of deionized water. All elution buffers and wash/diluent solution contained <0.05% sodium azide as a preservative, maintained at room temperature (15-30 °C), stable for 60 days, and mixed gently by inverting before use. Installing a New Reorder Pack Lot was done according to the manufacturer's package insert.

Whole blood primers

Whole blood Primers are lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. To prepare the whole blood primer 1.0 mL of deionized water was added to each vial, then allowed to stand for 10 minutes at 15-30 °C. The Whole blood Primer was used at the beginning of each run to condition the cartridge for analysis. The Whole blood Primer is stable for 21 days when stored at 2-8 °C.

Hemoglobin A2/F calibrator

The calibrator vials contain lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. To reconstitute the calibrator 10 mL of Calibrator diluent which is composed of deionized water with <0.05% sodium azide as a preservative, was used.

Quality controls

A set of normal (HbF: 1-2%, HbA_2: 1.8-3.2%) and abnormal (HbF: 5-10%, HbA_2 4-6%) controls were processed at the beginning and end of each group of each run of patient specimens.

Bio-Rad Lyphochek Hemoglobin A_2 controls were diluted 1:200 before analysis by pipetting 1.0 mL of Wash/Diluent solution into a labeled 1.5 mL vial, followed by 5 uL of the reconstituted control. Each control vial was capped and mixed thoroughly.

High-performance liquid chromatography (HPLC)

Ion exchange high-performance liquid chromatography (HPLC) was the basic principle of hemoglobin electrophoresis used in this study. The samples are automatically mixed and diluted on the VARIANT II Sampling Station (VSS) and injected into the analytical cartridge. The VARIANT Il Chromatographic Station (VS) dual pumps deliver a programmed buffer gradient of increasing ionic strength to the cartridge, where the HbA/F are separated based on their ionic interactions with the cartridge material. The separated HbA/F then passes through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. An additional filter at 690 nm corrects the background absorbance.

Data processing and analysis

The raw data gathered from each study is reduced by the VARIANT I Clinical Data Management (CDM"') program. The calibrated HbA/F readings are adjusted using a one-level correction. For each sample, DM produces a sample summary and a chromatogram. Based on their typical retention periods, frames have been created for the most prevalent hemoglobin to help with data analysis. Every type of hemoglobin has a distinctive holding period. By using the hemoglobin (HbA2/F) calibrator, minor variations in the separation effectiveness of individual analysis tubes are rectified.

Statistical analysis

Prism GraphPad software was used to apply the statistical analysis, and 0.05 p.value was used to indicate statistical significance indication.

RESULTS AND DISCUSSION

Abnormal hemoglobin electrophoresis

The analysis of 4009 samples resulted in the creation of chromatograms by noting retention times and area percentages of additional peaks and windows for structural variations, as well as HbA2 and F concentrations for beta-thalassemia. Each chromatogram displays the peaks of the hemoglobins A0, A2, and F as well as the windows C, D, and S and the two secondary peaks P2 and P3. Numerous hemoglobin variants eluted within the same frame, and they were provisorily identified by retention time and area percentage while considering the patients' racial backgrounds.

Other relevant tests were done, for example, the sickling test as supporting evidence of Hb S. Family study was carried out whenever possible and correlation with findings of Hb electrophoresis result was done in a few cases.

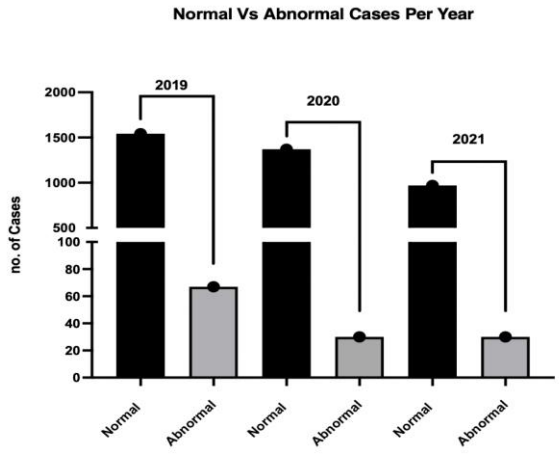

Figure 1. The total number of normal and abnormal hemoglobin cases for each year.

Figure 1 shows the total number of normal hemoglobin and abnormal cases for each year. In 2019 the total number of normal hemoglobin was 1542 subjects and the total number of abnormal hemoglobin was 67 subjects. In 2020 the total number of normal hemoglobin was 1370 subjects and the total number of abnormal hemoglobin was 30 subjects. In 2021 the total number of normal hemoglobin was 970 subjects and the total number of abnormal hemoglobin was 30 subjects.

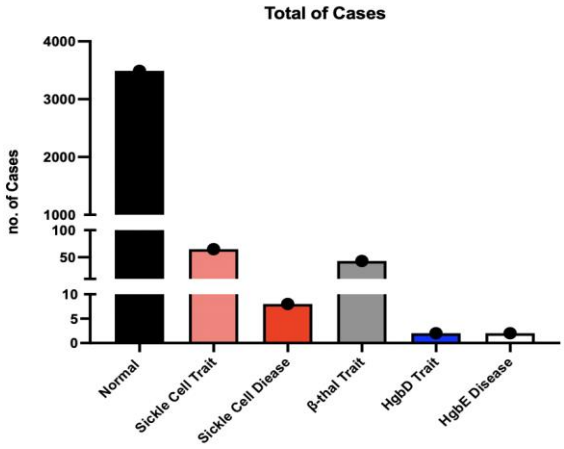

Figure 2. The total number of normal hemoglobin and abnormal hemoglobin diseases during 3 years.

Figure 2 shows the total number of normal hemoglobin and abnormal hemoglobin diseases during 3 years. The total number of normal hemoglobin was 3882 subjects, sickle cell trait was the most common hemoglobin disease accounting for 66 subjects (n=66). This is followed by the B-thal trait accounting for 50 subjects (n=50), followed by sickle cell disease accounting for 9 subjects (n=9), followed by hemoglobin D trait accounting for 1 subject (n=1), and hemoglobin E disease accounting for 1 subject (n=1).

The pattern of sickle cell disease and trait

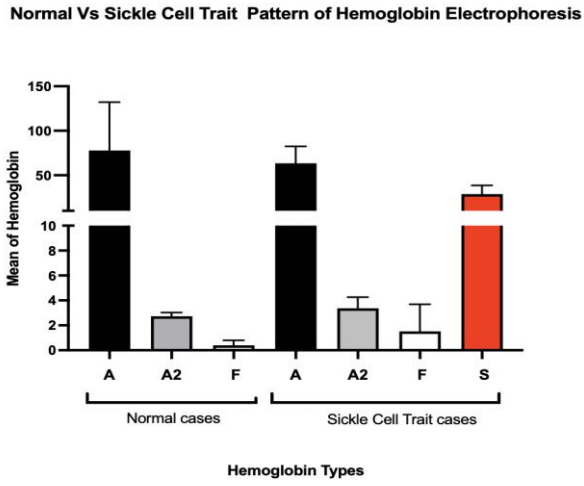

Figure 3. Hemoglobin pattern in sickle cell trait cases.

Figure 3 shows the hemoglobin pattern in sickle cell trait cases. The mean value of hemoglobin S in Sickle cell trait cases was 28.8±9.8 SD. The mean value ± SD of hemoglobin type A in normal subjects was 77.7±54.2 compared to hemoglobin type A in sickle cell trait cases (63.3±19.1). The mean value ± SD of hemoglobin type A_2 in normal subjects was 2.7±0.3 compared to hemoglobin type A_2 in sickle cell trait cases (3.4±0.9). The mean

value ± SD of hemoglobin F in normal subjects was 0.4 ± 0.4 compared to hemoglobin F in sickle cell trait (1.5±2.2).

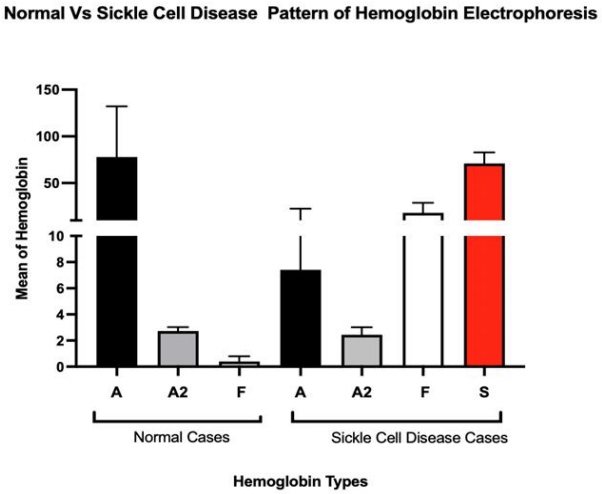

Figure 4. Hemoglobin pattern in sickle cell disease cases.

Figure 4 shows the hemoglobin pattern in sickle cell disease cases. As shown in **Figure 4** the mean value of hemoglobin S in Sickle cell disease cases was 70.9±11.9. The mean value ± SD of hemoglobin type A in normal subjects was 77.7±54.2 compared to hemoglobin type A in sickle cell disease cases (7.4±15.1). The mean value ± SD of hemoglobin type A_2 in normal subjects was 2.7±0.3 compared to hemoglobin type A_2 in sickle cell disease cases (2.4±0.6). The mean value ± SD of hemoglobin F in normal subjects was 0.4±0.4 compared to hemoglobin F in sickle cell disease cases (18.1±10.6).

The pattern of β - thalassemia trait and hemoglobin E disease

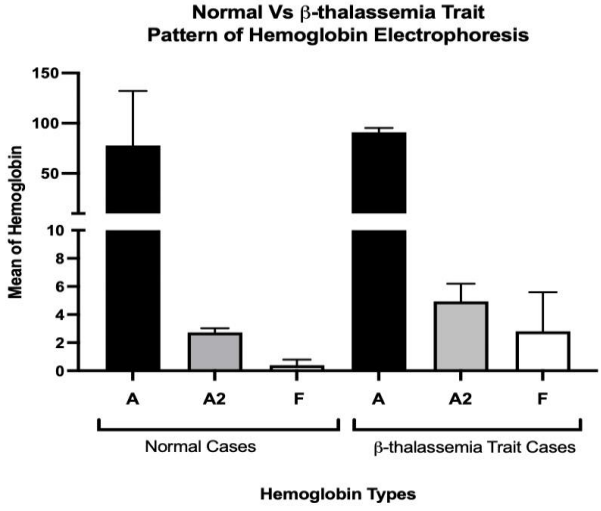

Figure 5. Hemoglobin pattern in B-thal trait cases.

Figure 5 shows the hemoglobin pattern in B-thal trait cases. The mean value ± SD of hemoglobin type A in normal subjects was 77.7±54.2 compared to hemoglobin type A in B-thalassemia trait cases (91.0±4.3). The mean value ± SD of hemoglobin type A2 in normal subjects was 2.7±0.3 compared to hemoglobin type A2 in B-thalassemia trait cases (4.9±1.3). The mean value ± SD of hemoglobin F in normal subjects was 0.4 ± 0.4 compared to hemoglobin F in B-thalassemia trait cases (2.8±2.8).

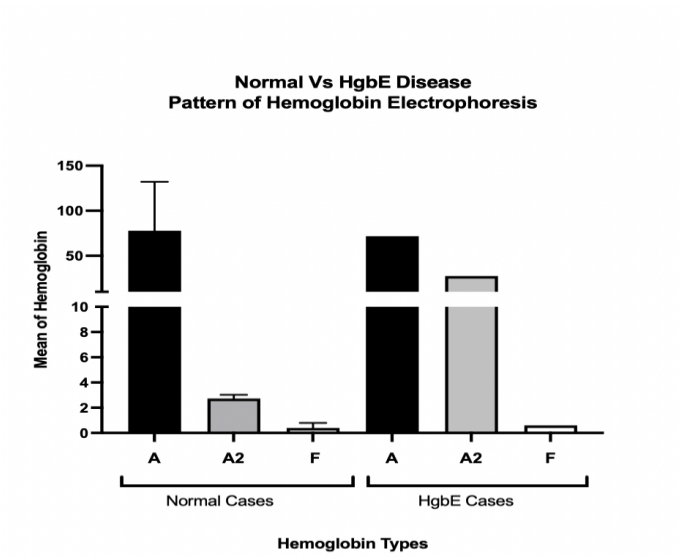

Figure 6. Hemoglobin pattern in hemoglobin E disease case.

Figure 6 shows the hemoglobin pattern in the hemoglobin E disease case. The mean value ± SD of hemoglobin type A in normal subjects was 77.7±54.2 compared to hemoglobin type A in hemoglobin E disease case (71.8±0.0). The mean value ± SD of hemoglobin type A2 in normal subjects was 2.7±0.3 compared to hemoglobin type A2 in the hemoglobin E disease case (27.6±0.0). The mean value ± SD of hemoglobin F in normal subjects was 0.4±0.4 compared to hemoglobin F in the hemoglobin E disease cases (0.6±0.0).

The pattern of hemoglobin in D trait case

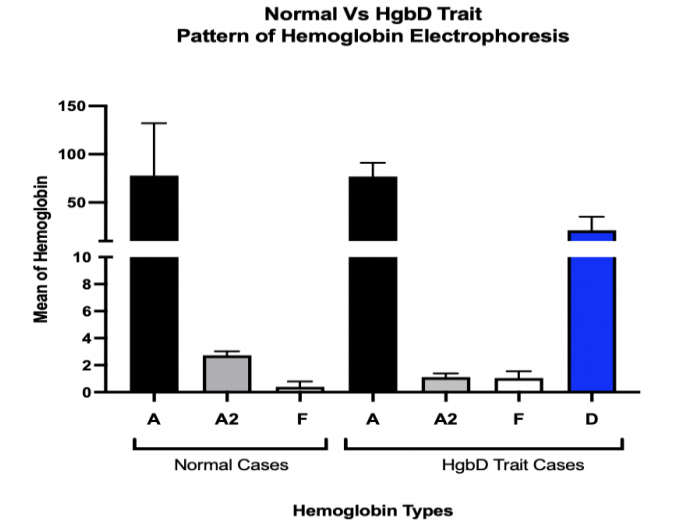

Figure 7. Pattern of Hemoglobin in D trait case.

Figure 7 shows a pattern of hemoglobin in the D disease case. The mean value ± SD of hemoglobin D in the D trait case was 21.1±14.1. The mean value ± SD of hemoglobin type A in normal subjects was 77.7±54.2 compared to hemoglobin type A in the D trait case (76.7 ±14.3). The mean value ± SD of hemoglobin type A2 in normal subjects was 2.7±0.3 compared to hemoglobin type A2 in the D trait case (1.1±0.3). The mean value ± SD of hemoglobin F in normal subjects was 0.4±0.4 compared to hemoglobin F in the D trait case (1.05±0.5).
Hemoglobinopathies, especially β-thalassemia and sickle cell disorders, are common in Saudi Arabia with varying frequencies in different regions of the country. Information about the prevalence of hemoglobin disorders in Saudi Arabia is patchy and probably underestimated, but studies have reported that hemoglobin disorders are a relatively

common genetic disorder in this part of the world. The prevalence of thalassemia and sickle cell disease in Saudi Arabia varies significantly in different parts of the country, with the highest prevalence in the Eastern province, followed by the southwestern provinces [12-17].

In Alhamdan *et al*. study that analyzed the first three years of data from the screening program, 4.20% of the study population had sickle cell trait, 0.26% had sickle cell disease, 3.22% had thalassemia trait, 0.07% had thalassemia disease. Both diseases were focused mainly in the eastern, western, and southwestern parts of the country. Among the 207,333 couples who were issued certificates for matching, 2.14% were declared high risk. Among the 2,375 high-risk couples contacted by telephone, 89.6% married each other, despite the known high-risk status [18].

In the Alsaeed *et al*. study, Secondary data analysis was obtained from the premarital screening and genetic counseling program (PMSGC) and included 12,30,582 individuals from February 2011 to December 2015.The highest rate for both β-thal and SCD was observed in the Eastern and Southern regions [10]. Studies from other Arab countries showed that the frequency of β-thal trait in general falls within the range of 2.0–10.0% [8, 9, 11].

the present study assessed the distribution of Hemoglobinopathies such as β-thalassemia and sickle cell disorders in the Saudi population of Al Majmaah City, Saudi Arabia. In this study, we used clinical data from the premarital screening center of Al-Majmaah for analysis. This study primarily involved the human subjects (who came for premarital screening) of Al Majmaah City, which is located in the central region (Riyadh Province) of Saudi Arabia. The current study showed that the total number of abnormal hemoglobin fractions on cation exchange-HPLC (CEHPLC) was seen in 127 cases. Sickle cell trait was the predominant genetic hemoglobin disorder accounting for 66 cases of the total cases. This was followed by the Beta-thalassemia trait in 50 cases, sickle cell disease in 9 cases, HgbE disease in 1 case, and HgbD trait in 1 case.

In our study, the majority of the positive cases were carriers of sickle-cell trait and b-thalassemia. Several other studies reveal that in most parts of Saudi Arabia, [4, 10, 18, 19] the most common Hb abnormality detected in this study was that of Sickle cell trait and β thalassemia trait. Therefore, there is a need for further prospective studies that document the prevalence and clinical epidemiology of Hb disorders in different areas of Saudi Arabia to help predict disease severity and describe the problems currently faced by affected patients in Saudi Arabia.

CONCLUSION

In our study, the majority of the positive cases were carriers' sickle-cell trait and b-thalassemia, several other studies reveal that in most parts of Saudi Arabia, Sickle cell trait and β thalassemia trait were the most common Hb disorders. There is a need for further prospective studies that document the prevalence and clinical epidemiology of Hb disorders in different areas of Saudi Arabia to help predict disease severity and describe the problems currently faced by affected patients in Saudi Arabia.

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CONFLICT OF INTEREST : None

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ETHICS STATEMENT : This study was approved by the Ethical Committee, King Fahad Medical City, Ministry of Health, Kingdom of Saudi Arabia, approval number: IRB Log. Number: 19-644E. All procedures performed in this study were in accordance with the ethical standards as laid down in the Declaration of Helsinki as revised in 2013.

A written informed consent from participants was taken following local/national guidelines.

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