Role of premarital screening program in detection of hemoglobinopathies at Al Baha, Saudi Arabia

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ABSTRACT

Background: Hemoglobinopathies are prevalent in Saudi Arabia. The use of high-performance liquid chromatography (HPLC) for screening of these disorders has recently been undertaken. This study was conducted to estimate the prevalence of hemoglobinopathies and evaluate the role of premarital screening centers among residents of Al Baha province, Saudi Arabia.

Methods: This retrospective cohort study utilized the laboratory reports of couples attending the premarital screening centers for the screening and confirmation of hemoglobinopathies in Al Baha province, Saudi Arabia. We obtained data from four hospitals and three primary healthcare centers authorized to perform these screening tests.

Results: The current study screened and analyzed a total of 2,165 reports for suspected hemoglobinopathies. Only 121 (5.6%) reports showed abnormal hemoglobin (Hb) patterns, among which the sickle cell trait was the most common, representing 59.5% (72/121) of the reported cases. This was followed by the β-thalassemia trait, Hb Lepore, sickle cell disease, and β-thalassemia major, accounting for 32.2% (39/121), 3.3% (4/121), 2.5% (3/121), and 2.5% (3/121), respectively.

Conclusion: Sickle cell and β-thalassemia traits are the major hemoglobinopathies in Al Baha, Saudi Arabia. Premarital screening is an invaluable measure to prevent childbirth with severe Hb disorders. The use of HPLC in the screening process provides a rapid, reliable procedure for the identification of various Hb fractions.

Keywords: Al Baha, Saudi Arabia, hemoglobinopathy, screening, hemoglobin variant, high performance liquid chromatography.

Background

Hemoglobinopathies including thalassemia and hemoglobin (Hb) variants are common public health problems in Saudi Arabia. They involve a diverse group of disorders. The prevalence of thalassemia trait and sickle cell anemia in Saudi Arabia is approximately 0.05% and 4.50%, respectively. Sickle cell anemia and thalassemia are prevalent in the southern regions of Saudi Arabia, primarily due to consanguineous marriages [1-5]. Several studies have been carried out to estimate the prevalence and explore the genetic structure of hemoglobinopathies in Al Baha southern regions of Saudi Arabia [6-9]. Many countries have adopted preventive screening programs to reduce the incidence of hemoglobinopathies. Saudi Arabia started this program in 2001 and made it compulsory by 2004 [1,10,11].

Clinical manifestations of both disorders include anemia of variable severity and pathophysiology [12-14]. Laboratory evaluation is the main method for the evaluation of HbA, along with other variants’ levels and red cell indices [15].
The purpose of premarital screening programs is to identify asymptomatic carriers of blood cell disorders so that they are aware of and can understand the reproductive risks and the available options. Therefore, there is a critical need for a screening procedure that can discover maximum variants. High-performance liquid chromatography (HPLC) provides good separation and quantitation of different Hb variants, such as HbF and HbA₂ [11].

Our study was conducted to estimate the prevalence of hemoglobinopathies and explore the role of premarital screening centers among the residents of Al Baha, Saudi Arabia. These centers use the cation exchange HPLC as a diagnostic tool in the detection of hemoglobinopathies and correlate their Hb profile with hematological features.

Materials and Methods

Study design, setting, and date

This retrospective cohort study utilized the laboratory reports of couples attending the premarital screening centers in Al Baha Province, Saudi Arabia between June 2018 and 2022 to perform the premarital screening tests and confirmation of hemoglobinopathies. Al Baha Province has four hospitals and three primary healthcare centers authorized to perform these screening tests.

Eligibility criteria

According to the Ministry of Health Guidelines, premarital screening is designed to test couples planning to get married for common genetic blood disorders as well as infectious diseases including hepatitis B, C, and HIV/AIDS.

The study included all the suspected cases of hemoglobinopathies who were sent for HPLC/ electrophoresis provided that all the related laboratory records were available during the study period. Individuals with incomplete laboratory investigation data were excluded.

Sampling technique

About 2-3 ml of intravenous blood were collected on ethylene diamine tetra acetic acid as anticoagulant using disposable syringes and needles from each individual free of blood transfusions.

Hematological analysis

The Sysmex XP 300 system hematology analyzer (Sysmex Corporation, Kobe, Japan) was used to determine the peripheral cell count and red blood cell (RBC) indices including the RBC count, Hb%, hematocrit value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration using the standard procedures. Peripheral blood smears were prepared and stained with Leishman stain to ascertain the morphological changes in RBCs in addition to the sickling test (using sodium meta-bisulfite).

Hematological analysis results interpretation

- **β-Thalassemia carriers**: the RBCs count is relatively high, while MCV and MCH are markedly reduced (MCV: 60-70 fl and MCH: 19-23 pg). The RBC morphology is modified and typically includes microcystosis, hypochromia, variation in the size and shape of red cells (anisopoikilocytosis), target cells, and basophilic stippling.
- **In carriers of Hb Lepore**, the hematological picture is characterized by mild anemia (Hb: 11-13 g/dl), microcystosis (MCV: 70-75 fl), and hypochromia (MCH: 20-24 pg).

Hb electrophoresis for hemoglobinopathy screening

It was carried out with an automatic analyzer HPLC (Variant II™, β-thalassemia short program; Bio-Rad Laboratories, Hercules, CA) using the HbA₂/F/A1c dual program kit. This was used for quantification of HbA₂, HbF, and for recognition and quantitation of the Hb variants that may interact with β-thalassemia, such as HbS and Hb Lepore. The resultant electropherograms were assessed visually for pattern abnormality. Scanning densitometry was used to determine the relative concentration of individual Hb fraction.

Hb electrophoresis results interpretation

This was based on the fractions of HbA₂ and HbF. The normal range for HbA₂ is between 1.7% and 3.2%, while that for HbF is usually less than 1.5% of the total Hb.
- **HbA₂ between 4.0% and 7%** indicates β-thalassemia carriers.
- **HbA₂ between 3.2% and 3.8%** is considered borderline and needs further investigation.
- **HbA > HbS, HbA > 50%-60%, and HbS 30%-40%** suggest sickle cell trait.
- **HbS 80%-100%, HbA₂ < 3.5%** with increased HbF suggest sickle cell anemia.
- **An abnormal fraction between 5% and 15% of the total Hb with reduced HbA₂ levels** (approximately 2%) and mildly increased HbF (2%-5%) suggest carriers of Hb Lepore.

Data analysis

The collected data from the laboratory reports were used to construct a central database. Data were analyzed using the Statistical Package for the Social Sciences software (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). The mean and the SD were calculated for the age and red cell parameters. Categorical variables were expressed as frequencies and percentages.
Premarital screening of hemoglobinopathies at Al Baha

Ethical considerations

This study obtained approval from the Scientific Research and Ethics Committee of the Faculty of Medicine, Al Baha University, Saudi Arabia (Approval ID: REC/PAT/BU-FM/2022/42).

Results

According to the data retrieved from laboratory records of HPLC-processed samples, a total of 2,165 reports were screened and analyzed for suspected hemoglobinopathies in the current study. The 2,165 analyzed laboratory reports were grouped according to age, with most of them (72.6%) falling in the age group of 18-32 years. Females were predominant, constituting more than half of the participants (56.4%), with an average age of 23.6 ± 5.4 years, while males constituted 43.6% with an average age of 25.8 ± 8.5 years (Table 1).

Most participants (94.4%) were normal, while only 121 (5.6%) showed abnormal Hb patterns, among which, the sickle cell trait constituted the most common abnormality, accounting for 59.5% (72/121) of these cases. This was followed by the β-thalassemia trait, Hb Lepore, sickle cell disease, and β-thalassemia major, which accounted for 32.23% (39/121), 3.31% (4/121), 2.49% (3/121), and 2.49% (3/121), respectively (Figure 1).

Table 3 shows that out of 121 hemoglobinopathy patients, 41 (60.29%) female patients were diagnosed with sickle cell trait compared to 31 (58.49%) male patients while 41 (60.29%) female patients were diagnosed with sickle cell trait, 0.26% had sickle cell disease, 3.22% had thalassemia minor. These findings were comparable to earlier reports from several regions of Saudi Arabia [10,16].

Discussion

Hemoglobinopathies are prevalent among Saudi citizens all over the country, with the highest frequency in the eastern region followed by the southwestern region. This might be due to the increased migration of people from and to these regions [3].

Our study aimed to explore the premarital screening and genetic counseling services among the residents of Al Baha, Saudi Arabia, and to assess their effect on the prevalence of hemoglobinopathies as well as their laboratory characterization.

Among the 2,165 analyzed laboratory reports of couples attending the premarital screening centers, 94.4% had normal Hb, while only 5.6% shows abnormal Hb patterns. The majority of the study participants were in the age group of 18-32 years, with female predominance constituting more than half of the participants (56.4%), with an average age of 23.6 ± 5.4 years. Males constituted 43.6% of the participants, with an average age of 25.8 ± 8.5 years. Abnormal Hb variants in their heterozygous states are clinically insignificant. Saudi cultural practices promote consanguineous marriages, thus homozygous variants may increase the likelihood of clinically manifest diseases.

The current study showed that Al Baha, Saudi Arabia had a high prevalence of sickle-cell disease and β-thalassemia accounting for 59.5% (72/121) and 32.3% (39/121), respectively. The findings of differences in sickle cell disease and β-thalassemia were comparable to earlier studies on abnormal Hbs in Saudi Arabia [10,16].

These findings were comparable to earlier reports from several regions of Saudi Arabia. Alhamdan et al. [10] found that 4.20% of the study population had sickle cell trait, 0.26% had sickle cell disease, 3.22% had thalassemia trait, and 0.07% had thalassemia disease. The researchers in this study analyzed data from the first 3 years of the screening programs in the eastern, western, and southwestern regions of Saudi Arabia.

Another research work [17] investigated hemoglobinopathies among Saudi adults at Taif city from October 2017 to August 2018. The study revealed comparable findings to our report, where 59% of the participants had heterozygous HbS as a major abnormality, and 20% presented with beta thalassemia minor. These findings were more or less similar to those recently observed by Ghazi et al. [18].

Table 1. Age distribution of participants attending premarital screening.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Males (n = 944)</th>
<th>Females (n = 1,221)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year, range (%)</td>
<td>18-50 (43.6)</td>
<td>16-60 (56.4)</td>
</tr>
<tr>
<td>Age, year, mean ± SD</td>
<td>25.8 ± 8.5</td>
<td>23.6 ± 5.4</td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-22, year, n (%)</td>
<td>72 (7.63)</td>
<td>395 (32.35)</td>
</tr>
<tr>
<td>23-27, year, n (%)</td>
<td>153 (16.21)</td>
<td>412 (33.74)</td>
</tr>
<tr>
<td>28-32, year, n (%)</td>
<td>317 (33.58)</td>
<td>223 (18.26)</td>
</tr>
<tr>
<td>33-37, year, n (%)</td>
<td>212 (22.46)</td>
<td>128 (10.48)</td>
</tr>
<tr>
<td>38-42, year, n (%)</td>
<td>143 (15.15)</td>
<td>33 (2.7)</td>
</tr>
<tr>
<td>&gt; 43, year, n (%)</td>
<td>47 (4.98)</td>
<td>30 (2.46)</td>
</tr>
</tbody>
</table>

n: number; SD: standard deviation.
Another study conducted by Alsaeed et al. [3] about the distribution of hemoglobinopathy disorders in Saudi Arabia based on data from the premarital screening and genetic counseling program 2011-2015 revealed that the highest rates for both β-thalassemia and sickle cell disease were observed in the eastern and southern regions of Saudi Arabia, and the overall prevalence rate per 1,000 population for sickle cell disease was 49.6 (45.8 for the trait and 3.8 for the disease) and 13.6 for β-thalassemia (12.9 for the trait and 0.7 for the disease). On the contrary, our study reported a much lower prevalence of β-thalassemia than earlier studies conducted by Alhamdan et al. [10], El-Hazmi and Warsy [19], and Mir et al. [15].

Regarding the hematological data among the study group, all those with sickle cell heterozygous showed positive sickling test, and most of them had microcytic hypochromic blood picture, which might be due to iron deficiency that has been frequently reported in carriers with sickle cell disease. β-thalassemia trait was the second most common abnormal Hb variant in our study, and the participants had microcytic hypochromic red cells with normal or slightly reduced Hb and raised RBC count.

**Limitation**

The study was conducted at the premarital screening centers of Al Baha, which could not represent the entire population.
population of Al Baha province. Therefore, the actual prevalence of thalassemia and other hemoglobinopathies could not be accurately evaluated. Despite this concern, the present study discloses a public health consideration in exploring this important issue in Al Baha region.

Conclusion

The study gives an outline of the magnitude and spectrum of hemoglobinopathies in Al Baha region, Saudi Arabia. A routine, compulsory premarital screening program is crucial for identification and prevention of high-risk marriages. It can be determined from the study that HPLC is a useful chromatographic technique for the diagnosis of hemoglobinopathies.

List of abbreviations

Hb  hemoglobin
HPLC  high-performance liquid chromatography
MCH  mean corpuscular hemoglobin
MCV  mean corpuscular volume
n  number
RBC  red blood cell

Authors’ contributions

All authors shared in the study conception as well as the study design. Material preparation, data collection, and analysis were carried out by Mujib Mesfer M. Alzahrani and Abdulrahman Salem F. Alghamdi. The first draft of the manuscript was prepared by Khalid Ali S. Alzahrani and Abdulrhman Saleh A. Alzhrani. The manuscript was revised by Abuobaida E. E. Abukhelaif. All authors have read and approved the final manuscript.

Acknowledgment

We acknowledge the technical staff and doctors working at the Premarital Screening Centers located at Al Baha province for their extended cooperation.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (register no: REC/PAT/BU-FM/2022/42, date: 27-12-2022.).

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References

13. Mosca A, Paleari R, Ivaldi G, Galanello R, Giordano PC. The role of haemoglobin A(2) testing in the diagnosis of
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