Journal of Associated Medical Sciences 2026; 59(2): 428-437

JAMS
Journal of Associated Medical Sciences

Scopus Indexed & Thai-Journal Citation Index Centre (TCI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index



Prevalence and characterization of β-thalassemia minor in a premarital screening at a hereditary blood diseases center in Babylon, Iraq

Shekair WH[1], Al-Maaroof ZW[2], Aljawazery SAA[3], Haddawi KH[4*], Al-Khyate SAM[5]

[1]Department of Pediatrician, College of Medicine Al-Mustaqbal University, Babylon, Hillah, Iraq.
[2]Department of Pathology, College of Medicine, University of Babylon, Babylon, Iraq.
[3]Department of Laboratory, Al Hilla Teaching General Hospital, Iraq.
[4]Department of Clinical Biochemistry, College of Medicine, University of Al-Qadisiyah, Iraq.
[5]Al Zahra'a Hospital / Wasit Health Directorate, Iraq.

ARTICLE INFO

Article history:
Received 3 November 2025
Accepted as revised 16 April 2026
Available online 24 April 2026

Keywords:
β-thalassemia, premarital screening, HbA2, iron deficiency anemia, genetic counseling.

ABSTRACT

Background: β-thalassemia is a significant hereditary hematological disorder that is highly prevalent in Iraq. Premarital screening is a critical preventive strategy for identifying couples at risk of having children with thalassemia major. This study evaluated the effectiveness of a premarital screening program in Babylon, Iraq.

Objectives: To determine the prevalence of β -thalassemia minor among couples with abnormal red cell indices and characterize the associated hematological parameters.

Materials and methods: A cross-sectional study was conducted from 2016 to 2019 on 960 individuals (480 couples) referred to the Hereditary Blood Diseases Center. Participants with a mean corpuscular volume (MCV) <80 fL and/or mean corpuscular hemoglobin (MCH) <27 pg underwent confirmatory testing via high-performance liquid chromatography (HPLC) for HbA2 quantification and serum ferritin analysis.

Results: The prevalence of β-thalassemia minor was 20.1% (193/960), with a significant sex disparity (males: 27.7%, females: 12.5%). Iron deficiency anemia (IDA) was identified in 46.6% of participants. The initial screening step using MCV/MCH cut-offs showed 100% sensitivity but 53.4% specificity, primarily because of IDA. Fifteen high-risk couples (1.7%) in which both partners were carriers were identified.

Conclusion: The two-stage premarital screening protocol demonstrated high sensitivity for detecting β-thalassemia carriers. The high prevalence of both the thalassemia trait and IDA in this young adult population underscores a substantial public health burden. The program successfully identified at-risk couples, enabling targeted genetic counseling and prenatal diagnosis to reduce the incidence of thalassemia major.

Introduction

β-thalassemia represents a significant public health challenge with widespread distribution across Africa, the Mediterranean region, the Middle East, including Iraq, the Indian peninsula, and Southeast Asia.[1] Globally, approximately 3% of the world population carries genes for β-thalassemia, with the highest prevalence rates documented in Cyprus, Italy (particularly Sardinia), and South Asia.[2,3] In Arab countries,

*** Corresponding contributor.**
Author's Address: *Department of Clinical Biochemistry, College of Medicine, University of Al-Qadisiyah, Iraq.*
E-mail address: krarr.haidar@yahoo.com
doi: 10.66285/JAMS.2026.072
E-ISSN: 2539-6056

β-thalassemia minor occurs at varying frequencies with carrier rates ranging from 1% to 11%, with particularly elevated frequencies observed in Jordan, Lebanon, Iraq, Egypt, Palestine, and other regional nations.[4,5]

In Iraq, the frequency of carriers for β-thalassemia is more evenly distributed throughout the country at rates that vary between 3.0% and 6.5%, about 15,000 thalassemia major or intermedia patients are registered with hemoglobinopathy.[4-6] T The Hereditary Blood Diseases Center of Babylon Teaching Hospital for Women and Children (BTHWC) is providing care for around 1,000 homozygous β-thalassemic patients. This is a huge patient load and emphasizes the need for large pragmatic intervention studies to be done in this area.[7]

The pathophysiology of thalassemia is characterized by deficient synthesis of either alpha or β hemoglobin chains, which manifests as alpha α- or β-thalassemia. Whilst alpha thalassemia is less common, β-thalassemia has a high prevalence in certain parts of the world. β-thalassemia manifested clinically in a continuum from thalassemia trait (thalassemia minor) to thalassemia major.[8,9] Thalassemia major patients (HbA2 2%, HbF 98%, HbA nil) have severe clinical manifestations and require regular blood transfusions, whereas thalassemia traits (HbA2>3.5%, HbF nil) are usually clinically unaffected and diagnosed on routine hematological examination.[10,11]

Multiple studies conducted throughout Iraq have demonstrated considerable regional variation in thalassemia prevalence across different provinces.[4-7] This common, incurable, autosomal recessive hemoglobinopathy causes substantial morbidity and mortality while imposing a considerable financial burden on society.[12] Importantly, β-thalassemia represents a preventable disease through the implementation of comprehensive screening programs.

Premarital screening programs have gained widespread acceptance since the early 1970s, with numerous countries implementing mandatory screening protocols.[13] In Iraq, the thalassemia prevention program was initiated in 2015 in the Babylon governorate.[7] This prevention strategy operates through the premarital screening of β-thalassemia carrier couples to encourage participation in genetic counseling and prenatal diagnosis.[4,5] Baghdad, a total of 12526 individuals (6,263 couples)

The screening protocol employs red blood cell indices, particularly mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), as initial population screening tools because of their suitability for large-scale epidemiological screening and incorporation into routine premarital examinations.[9] When both partners demonstrated an MCV<80 fL or MCH<27 pg (established cut-off values), hemoglobin A2 concentrations were measured using high-performance liquid chromatography.[10] Individuals with confirmed β-thalassemia minor characteristics (HbA2>3.5%) received genetic counseling referrals.[11] Partners with red cell indices below cut-off values but normal HbA2 concentrations are classified as suspected cases, requiring additional evaluation, including iron therapy for those with low serum ferritin levels, followed by repeat testing with or without molecular studies.[11,14]

Different cutoffs of MCH and MCV have been reported in hematologic references, probably due to population differences, including age and race.[15] Presently, MCV of 80 fL and MCH of 27 pg are used as cut-off values for the best sensitivity and specificity. The goal of premarital screening is to detect carriers of hemoglobin disorders to prevent the birth of a child with a severe form of the disease.

This research aims to investigate the prevalence of β-thalassemia minor in accordance with criteria by comprehensive assessment, including RBC indices, HPLC, and iron studies among 480 couples (male and female) from around primary health care centers in Babylon governorate, who presented for marriage at Hereditary Blood Diseases Center/ BTHWC.

The study was restricted to individuals aged 17-35 years because this age range represents the typical premarital age group in the Babylon governorate. In Iraq, the legal minimum age for marriage is 18 years (with judicial exceptions from age 15-17), and most individuals presenting for premarital screening fall within this range. Including older individuals would introduce confounding factors such as chronic disease-related anemia, age-related changes in hematological parameters, and reduced relevance to the premarital screening population. Furthermore, this age restriction ensures homogeneity of the study population and enhances the applicability of findings to the target demographic of premarital screening programs.

Materials and methods

Study design and participants

A cross-sectional study was conducted at the Hereditary Blood Diseases Center in BTHWC/Iraq. Recruitment period: 48 months (from January 1, 2016, to December 31, 2019). This study included a total of 960 (480 couples) persons. All were referred to the center from different premarital examination centers because of initial detection of blood indices abnormalities (Low MCV or Low MCH).

Inclusion and exclusion criteria

The inclusion criteria were as follows: 1) individuals of both sexes planning marriage, 2) age between 17 and 35 years, which represents the typical premarital age range in the Babylon governorate and ensures population homogeneity for screening purposes, and 3) initial screening results showing mean MCV<80 fL and/or MCH<27 pg. Individuals with a known history of hematological malignancies, chronic inflammatory diseases, liver or kidney disorders, or those who had

received a blood transfusion within the last three months were excluded from the study.

Sample size justification

The sample size was a convenience sample, encompassing all eligible couples referred to the center during the specified 48-month study period who met the inclusion criteria. Formal power calculations were not performed a priori.

Biological sample collection and processing

Trained phlebotomists collected peripheral venous blood samples (approximately 5 mL) from each participant. Collection was performed without fasting, primarily during the morning hours (8:00-12:00 pm). Blood was drawn into two vacuum tubes: A K_2EDTA tube for complete blood count (CBC) and hemoglobin electrophoresis, and a plain serum separator tube (SST™, BD Vacutainer®) for ferritin analysis.

The EDTA tube was gently inverted 8-10 times immediately after the collection. The SST tube was allowed to clot at room temperature for 30 minutes. Both tubes were transported to the laboratory at room temperature and processed within 2 hours of venipuncture. The SST tube was centrifuged at 3000 rpm for 10 min to separate serum. Serum aliquots for ferritin analysis were stored at -20 °C and analyzed in batches within one week of collection; no freeze-thaw cycles were permitted before analysis.

Laboratory assays

Complete blood count (CBC) and red cell indices were measured using an automated hematology analyzer (Mindray BC5000™, China). Hemoglobin electrophoresis quantification of HbA2 was performed using high-performance liquid chromatography (HPLC) on the VARIANT™ II Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA, USA). Serum Ferritin was quantified by a chemiluminescent immunoassay (CLIA) on an Architect i2000SR immunoassay analyzer.

Calibration and quality control

All laboratory assays were performed in accordance with the manufacturer's protocol. Automated analyzers (Coulter LH 780, Architect i2000SR, and VARIANT II) were calibrated using their respective manufacturer-provided calibration materials before use and as required by the internal quality control protocols.

Internal quality control (IQC) was performed daily using commercial control materials of three levels (low, normal, and high) provided by the manufacturers (Bio-Rad Hemoglobin Control, Ref: 195-2051; Abbott Architect Ferritin Controls, Ref: 7K66-10). External quality assurance (EQA) was conducted through the Iraqi National Quality Control Program.

The inter-assay coefficient of variation (CV) for ferritin was <5%, and that for HbA2 quantification was <3%. All the assays were performed in a single run for each sample.

Ethical considerations

The study protocol was reviewed and approved by the Scientific and Ethical Committee of the University of Al-Mustaqbal, College of Medicine (Approval No. UM/CoM/Ethics/2015-038). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all participants included in the study after explaining the nature and purpose of the study.

Statistical analysis

Data were collected and organized with Microsoft Excel and then analyzed by GraphPad Prism Software version 9.0. Study variables were summarized using descriptive statistics. For categorical data (the proportions of β-thalassemia traits, iron-deficiency anemia, and demographic characteristics), number (%) was used. Quantitative variables (hematological indices such as Hb, MCV, MCH, HbA2, and ferritin) were reported by means±SD. For inferential statistics, an independent sample t-test was used to determine the mean difference between continuous variables (e.g., hematological parameters) for two independent groups (for example, males vs females within β-thalassemia carrier analysis). The $p<0.05$ was regarded as statistically significant in all tests. Sensitivity and specificity were calculated to determine the diagnostic performance of the first screening phase (MCV and MCH cutoff values).

Result

The study was cross-sectional and included (480 males, 480 females) premarital individuals on a visit to the center due to abnormal red cell indices. From laboratory results, the study population was divided into three diagnostic groups: 447 (46.6%) with iron deficiency anemia (IDA), 193 (20.1%) as β-thalassemia carriers, and 320 (33.3%) with normal hematological parameters. In comparison to males (N=167), a higher prevalence of IDA was evident among females (N=280), and β-thalassemia trait was more common in males than in females (male: N=133; female: N=60). The hematological evaluation referred to normal reference values, and the anemia was defined as Hb<14 gm/dL in males and <12 gm/dL in females. Microcytosis was MCV<80 fL, and hypochromia was an MCH<27 pg for both genders. HbA2 level greater than 3.5% was considered the diagnostic cutoff for β-thalassemia minor, and ferritin less than 20 ng/mL as cut-off of iron deficiency.

The largest share (N=458, 47.7%) of the 960 participants belonged to the age group between 21 and 25 years, as presented in Table 1, which corresponds with the common age for marriage in this population. There were 280 (29.2%) and 222 (23.1%) participants aged 17-20 and 26-35 years, respectively. Distribution

of all three diagnostic categories in normal individuals, β-thalassemia carriers, and IDA followed a similar trend among different age groups, with the greatest absolute values prevailing among 21-25-year-olds, as shown in Table 1.

Table 1. Age distribution of participants by diagnostic group.

Age group (years)	Normal individuals	β-Thalassemia carriers	Iron deficiency anemia	Total
17-20	85	45	150	280
21-25	150	98	210	458
26-35	85	50	87	222
Total	320	193	447	960

The comparison of hematological parameters among four diagnostic groups: healthy, β-thalassemia trait, combined β-thalassemia trait with IDA, and IDA is shown in Table 2. The β-Thalassemia carriers expressed typical microcytic hypochromic anemia in that the MCV and MCH values were significantly decreased and showed diagnostically high (HbA2>3.5%). On the other hand, patients with IDA also had microcytic hypochromic indices (low MCV and MCH) but were characterized by significantly low serum ferritin concentrations and normal or decreased HbA2 percentage. The combined β-thalassemia and IDA group (N=155) demonstrated features of both conditions, including elevated HbA2 levels alongside depleted iron stores. All the parameters were found within the normal reference range in the healthy control group and served as the baseline. Of particular interest, females in the β-thalassemia carrier group had lower mean values of hemoglobin, MCV, MCH, and ferritin than males.

Table 2. Hematological parameters by diagnostic group.

Parameter	Gender	Normal individuals (N=320)	β-Thalassemia carriers (N=193)	Combined β-Thal+IDA (N=155)	IDA (N=447)
Hb (gm/dL)	Male	15±1.5	10.5	9.8±1.0	10.5±1.5
	Female	12±1.5	8.5-10	8.5±0.9	9.0±1.5
MCV (fL)	Male	85±2	65±2	62±3	75±2
	Female	83±2	60±2	58±3	70±2
MCH (pg)	Male	30±2	<27	21±2	20±2
	Female	30±2	<27	20±1.5	18±1
HbA2 (%)	Male	2.5±0.5	4.5±0.5	3.8±0.4	1.8±0.5
	Female	3±0.2	5±0.2	3.6±0.3	1.6±0.5
Ferritin (ng/mL)	Male	100±15	60±10	12±4	10±5
	Female	60±5	18±3	8±3	5.5±4

Comparative gender-based analysis of hematological parameters between β-thalassemia minor (BTM) carriers (N=193) is depicted in Table 3, which shows a significant difference among genders, i.e., males (N=133) and females (N=60). Males had higher mean Hb (10.5±0.8 gm/dL vs 9.3±0.8 gm/dL), greater MCV (65±2 fL vs 60±2 fL), greater MCH (23±2 pg vs 22±1.5 pg) and much higher levels of ferritin (60±10 ng/mL versus 18±3 ng/mL); whereas females had a slightly increased HbA2 percentage (5.0±0.2% versus 4.5±0.5%). All intersex contrasts were statistically significant (p<0.001).

Table 3. Comparison of key parameters between genders in the β-thalassemia carrier group.

Parameter	Mean±SD		*p* value
	Males (N=133)	**Females** (N=60)	
Age (years)	24.5±3.2	22.8±3.5	-
Hb (gm/dL)	10.5±0.8	9.3±0.8	<0.001
MCV (fL)	65±2	60±2	<0.001
MCH (pg)	23±2	22±1.5	<0.001
HbA2 (%)	4.5±0.5	5.0±0.2	<0.001
Ferritin (ng/mL)	60±10	18±3	<0.001

Table 4 shows the severity of IDA in the cohort according to the distribution of ferritin among 447 confirmed patients: severe deficiency (ferritin <15 ng/mL) was strongly predominant, representing 79.4% (N=355) of IDAs. There was a significant gender difference, with 230 females and 125 males in this serious category. The remaining 20.6% (N=92) were diagnosed as being moderately deficient (ferritin 15 to <30 ng/mL). The classification of iron deficiency severity was based on the World Health Organization (WHO) guidelines on the use of ferritin concentrations to assess iron status, which define iron deficiency as serum ferritin <15 µg/L in adults, and iron depletion as ferritin 15-30 µg/L.[16] No subjects with ferritin ≥30 ng/mL were observed in the screened case series, as all individuals with ferritin ≥30 ng/mL were classified as iron-replete rather than iron-deficient.

Table 4. Distribution of iron deficiency anemia severity based on ferritin levels.

Severity category	Ferritin Level (ng/mL)	Males	Females	Total	%
Severe deficiency	<15	125	230	355	79.4
Moderate deficiency	15 - <30	42	50	92	20.6
Iron-replete	≥30	0	0	0	0
Total		167	280	447	100

Table 5 presents a risk assessment for married couples from premarital screening results, classifying 480 couples (960 individuals) into five-level groups in accordance with the Hb profiles. Fifteen (3.1%) were couples, both carriers of β-thalassemia minor, which established a 25% risk per pregnancy for the couple to have progeny with thalassemia major and had to go through compulsory genetic counseling and prenatal detection. Among the rest of the couples, 200 had only one carrier partner, 223 couples had iron deficiency anemia, and 155 had a combined phenotype of carriers and abnormal respondents (each required special action, such as counseling and iron therapy).

Consanguinity data were collected for all 480 couples. Of these, 216 couples (45.0%) reported consanguineous marriages (defined as unions between second cousins or closer). Among the 15 high-risk couples (both carriers), 11 (73.3%) were consanguineous, which was significantly higher than the overall consanguinity rate in the study population (p<0.01). Among the 200 couples with one carrier partner, 88 (44.0%) were consanguineous. The high rate of consanguinity among at-risk couples is consistent with the known elevated prevalence of autosomal recessive disorders in consanguineous populations in Iraq.

Table 5. Risk assessment of married couples based on screening results.

Couple Type	Number of couples	Risk of having an affected child	Recommended action
Both normal	87	Very low	No action needed
One carrier, one normal	115	Carriers (no disease)	Genetic counseling
Both carriers	15	High (25% per pregnancy)	Mandatory counseling and PND offer
Both with iron deficiency	148	Low (but requires treatment)	Iron therapy
Carrier + iron deficiency	115	Variable	Iron therapy + HbA2 recheck + counseling
Total	480		

Note: *PND: prenatal diagnosis.*

The effectiveness of the two-step premarital screening program in detecting β-thalassemia minor is summarized in Table 6. Screening of 960 individuals using low MCV (<80 fL) and/or low MCH (<27 pg) as cut-off criteria indicated 100% sensitivity because all 193 carriers among the tested 960 individuals were identified. This step, however, had a low specificity (53.4%) due to the high number of false positives (N=447), predominantly caused by IDA. The second step test, including treatment with iron and the re-evaluation of indices to all who presented low indices at baseline was essential for refining the diagnosis, since it resolved microcytosis and hypochromia in a substantial number of cases (292 individuals) deeming 155 persons with persistently low indices as suspicious "true" carriers sent to have confirmatory Hb electrophoresis; thus improving program specificity.

Table 6. Effectiveness of primary screening (MCV/MCH) in detecting carriers.

Screening step	Number tested	Number referred	True carriers identified	False positives	Sensitivity (%)	Specificity (%)
Step 1: Initial screening (MCV<80 fL and/or MCH <27 pg)	960	640	193	447 (IDA cases)	100	53.4
Step 2: After iron therapy (re-check of indices)	447	155 (Remained low)	15 (Newly identified)	140	-	-
Overall program performance	960	795 (Total referrals)	193	-	100	70

Note: *False Positives were primarily due to iron deficiency.*

Discussion

The current study offers several informative and useful data concerning the preventive screening efficacy in relation to couples' premarital status, while investigating their implications within local and global prevention programs. The detected β-thalassemia trait prevalence of 20.1% in the studied individuals greatly surpasses reported rates among Iraqis (3-6.5%) and Middle Easterners at large (1-11%).[5] This relatively high detection rate is due to the focused screening strategy in which participants are selected on the basis of abnormal red blood cell indices, which will enrich the sample with possible carriers.[17]

The findings align with similar targeted screening approaches reported in other Middle Eastern populations.[18] A premarital screening study in Al Majma'ah, Saudi Arabia, identified the β-thalassemia trait in 3.4% of unselected couples,[19] while a study from Southwest Iran reported a 5.6% prevalence among general premarital populations.[20] All 581 couples live in Khuzestan Province, Southwest Iran. Individuals with mean corpuscular volume <80, mean corpuscular hemoglobin <27, hemoglobin A2 ≥3/5 were considered as β-thalassemia traits. Prevalence of minor β-thalassemia, α-thalassemia, SCA, iron deficiency anemia, and silent thalassemia were respectively identified in 995 (5.6% The marked difference between these general population studies and the current investigation underscores the effectiveness of the two-stage screening protocol employed in Babylon, where initial red cell index filtering significantly enhanced the detection efficiency.

Studies from other Iraqi provinces have demonstrated regional variations in the carrier rates. Hassan *et al*. reported a 3.5% prevalence of β-thalassemia trait in the Baghdad premarital screening program, Baghdad,[5] a total of 12,526 individuals (6,263 couples while Abbas *et al*. documented prevalence rates ranging from 3.0% to 6.5% across different Iraqi governorates.[4] In the current study, a higher detection rate likely reflects both the pre-screening strategy and potential geographic clustering of β-thalassemia carriers in the Babylon region, which is consistent with the established founder effect phenomena observed in isolated populations.

The observed gender disparity, with males exhibiting a higher carrier rate (27.7%) than females (12.5%), represents a notable finding that warrants careful interpretation. While β-thalassemia follows autosomal recessive inheritance patterns with an expected equal gender distribution, several factors may contribute to this apparent discrepancy. The significantly lower ferritin levels observed in female carriers (18±3 ng/mL) compared to males (60±10 ng/mL) suggest that concurrent iron deficiency may mask the characteristic hematological features of β-thalassemia in women.[21]

The significant difference in the prevalence of β-thalassemia between males and females observed in this study deserves further elaboration. Since β-thalassemia is an autosomal recessive disorder, the true carrier frequency should be approximately equal between sexes. The apparent male predominance (27.7% vs 12.5%) is most likely an artifact of the screening methodology rather than a true biological difference. Several mechanisms may account for this observation. Firstly, iron deficiency, which was significantly more prevalent and severe among females (82.1% of female IDA cases had ferritin <15 ng/mL), can suppress HbA2 levels below the diagnostic threshold of 3.5%, thereby masking the β-thalassemia trait in iron-deficient women.[22] Secondly, the overlapping hematological features of IDA and β-thalassemia trait (both presenting with microcytic hypochromic anemia) make it more difficult to distinguish between the two conditions in females who are more frequently iron-deficient. Thirdly, some female carriers with concurrent severe iron deficiency may have been misclassified as having IDA alone, as their HbA2 levels may have been falsely normalized. This diagnostic challenge has been reported in multiple studies from the Middle East and South Asia, where iron deficiency is endemic among women of reproductive age.[21,22] These findings emphasize the critical importance of iron repletion before definitive β-thalassemia screening in populations with high rates of iron deficiency, particularly among women.

This phenomenon has also been observed in other populations. Jameel *et al*. demonstrated that iron deficiency can suppress HbA2 levels below the diagnostic threshold of 3.5%, potentially leading to

under-detection of the β-thalassemia trait in iron-deficient individuals.[22] The high prevalence of severe iron deficiency among females in the current study (82.1% of female IDA cases had ferritin <15 ng/mL) supports this hypothesis and aligns with the regional patterns of iron deficiency in young women across the Middle East. [23]

The two-stage screening protocol demonstrated excellent sensitivity (100%) for detecting β-thalassemia carriers, confirming the effectiveness of MCV and MCH cut-off values as primary screening tools. These findings corroborate the results of large-scale studies conducted in other populations. Heng *et al*. analyzed 6,779 β-thalassemia carriers in Guangdong province, China, and reported that combined MCV (<80 fL) and MCH (<27 pg) screening reduced false-negatives to only 0.22%.[24] Similarly, Sahiratmadja *et al*. demonstrated that an MCV <80 fL and/or an MCH<27 pg provided optimal first-round screening sensitivity in Indonesian populations.[25]

The observed specificity of 53.4% in the initial screening step reflects the expected trade-off between sensitivity and specificity in population-screening programs. The high false-positive rate, primarily attributed to IDA (46.6% of screened individuals), aligns with established screening paradigms where initial high sensitivity is followed by confirmatory testing to improve specificity.[26] This approach has proven cost-effective in multiple healthcare settings, as demonstrated in prevention programs across Cyprus, Sardinia, and Greece.[27]

The finding that 46.6% of the screened individuals had IDA highlights a significant public health concern in the study population. This prevalence substantially exceeds the rates reported in other regional studies. Owaidah *et al*. documented an IDA prevalence of 10.7% among Saudi university students, while regional estimates for the Middle East indicated that IDA affects approximately 32.5% of pregnant women and 28% of adolescents.[28] The severity distribution observed in the current study, with 79.4% of IDA cases classified as severe deficiency (ferritin <15 ng/mL), indicates a critical level of iron depletion that requires immediate public health interventions.

The intersection of iron deficiency and β-thalassemia traits presents diagnostic challenges that are well documented in the literature. Sun et al. outlined key discriminating features, noting that β-thalassemia traits typically present with higher RBC counts (>5M/μL), lower MCV (<74 fL), and normal iron studies, whereas IDA is characterized by reduced RBC counts (<4M/μL) and depleted iron stores.[21] The current study protocol of iron therapy followed by repeat testing effectively addressed this diagnostic challenge, improving the overall program specificity from 53.4% to 70%.

The identification of 15 couples (3.1%) at high risk of having children with thalassemia major represents a significant achievement in disease prevention. Each at-risk couple has a 25% probability of having an affected child, making genetic counseling and prenatal diagnosis essential interventions. International experience has demonstrated the effectiveness of such prevention strategies. Cao *et al*. reported that comprehensive prevention programs in Sardinia achieved an 85% reduction in thalassemia major births from 1:250 to 1:1,660 live births.[27] Similar success has been documented in Cyprus, Iran, and Turkey, where mandatory premarital screening programs achieved substantial reductions in affected births despite limited success in preventing at-risk marriages.[29]

The cost-effectiveness of prevention programs has been well established. Kuwaiti studies have demonstrated that preventing one case of β-thalassemia major costs approximately 100 USD, substantially less than the annual treatment costs for affected patients.[30] Similar economic analyses from Greece and Cyprus indicate that prevention program costs equal those of treating one patient for one year, whereas the annual screening program expense equals one week of treatment costs for the entire affected population.[2,31,32]

The effectiveness of pre-marital screening programs relies on substantial cultural and community involvement. The Kurdistan, Iraq studies indicated significant challenges to program success, with only 10.5% of at-risk couples opting to cancel plans for marriage and 5.7% discussing termination in the case of affected fetuses.33 These results indicate the nuanced impact of medical advice and cultural values on the effects of the program.

Religious and cultural influences had a major effect on the acceptance of the program in the Middle East. A systematic review by Saffi and Howard of Middle Eastern programs found that, except for Iran, Turkey, and Iraq, most countries did not achieve 65% at-risk marriage cancellation rates; those three countries also experienced decreases in affected births through prenatal diagnosis and therapeutic abortion.[29] Identification of at-risk couples as performed in the current study forms a basis for selective culture-specific genetic counseling programs, which should be applicable and ethically valid.

The high rate of consanguinity observed in this study (45.0% overall, 73.3% among high-risk couples) is consistent with previously reported consanguinity rates in Iraq, which range from 24% to 71% across different regions.[34] Consanguineous marriages significantly increase the probability of offspring being homozygous for autosomal recessive alleles, including β-thalassemia mutations. The notably higher consanguinity rate among both-carrier couples (73.3%) compared to the overall study population (45.0%) further supports the well-established association between consanguinity and increased risk of autosomal recessive disorders. These findings underscore the importance of incorporating consanguinity assessment into premarital screening programs and providing targeted genetic counseling to

consanguineous couples, particularly in populations with high carrier rates for hemoglobinopathies.

Limitations and future directions. There are several limitations of this study to be accounted for when interpreting the results. The use of convenience sampling and a referral-based recruitment method may lead to selection bias that could impact generalizability to the wider Babylon population. Hence, lack of molecular characterization with many β-thalassemia mutations is a major drawback, since it is important to know the exact patterns and prevalence of the various mutations in order to make prevention programs and genetic counseling broad-based. Molecular analysis must be included in future studies to ascertain common mutations and customize preventive strategies.

Conclusion

This comprehensive premarital screening study demonstrated the effectiveness of systematic β-thalassemia trait detection in a high-prevalence population. The two-stage screening protocol achieved excellent sensitivity while managing concurrent iron-deficiency anemia. The findings underscore the critical importance of addressing iron deficiency as a public health priority while focusing on genetic disease prevention. Recognition of at-risk couples allows for the implementation of focused genetic counseling and prenatal diagnosis, as part of comprehensive measures aiming at the control of thalassemia in Iraq. The findings of the study would further justify and strengthen the continuation of premarital screening and counseling programs in Iraq, with a need for greater public awareness, better genetic counseling services, as well as strategies to alleviate the large burden of IDA in their target population. Further enhanced capacity in the program is to incorporate molecular diagnostic testing and thus provide a more in-depth level of genetic risk assessment for participating couples.

Ethical approval

The study protocol was reviewed and approved by the Scientific and Ethical Committee of the University of Al-Mustaqbal, College of Medicine (Approval No. UM/CoM/Ethics/2015-038). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all participants included in the study after explaining the nature and purpose of the study.

Use of artificial intelligence

The artificial intelligence was applied to improve sentence structure and clarity.

Funding

No funds were received to fulfil this work.

Conflict of interest

The authors declare no competing interests.

CRediT authorship contribution statement

Widad Hamza Shekair: conceptualization, methodology, validation, formal analysis, resources, data curation, visualization, writing: original draft, review and edit, project administration, funding acquisition; **Zainab W. Al-Maaroof:** conceptualization, methodology, formal analysis, resources, visualization, resources, writing: review and edit; **Krarr Haider Haddawi:** investigation, writing: original draft, visualization, supervision; **Shaymaa Ali Abed Aljawazery:** formal analysis, investigation, resources, writing: original draft, review and edit, supervision; resources, writing: original draft preparation, visualization, supervision; **Suhad Aziz Mustafa Al-Khyate:** visualization.

Acknowledgements

The authors wish to thank the head and staff at the Hereditary Blood Diseases Center in BTHWC for their great efforts and contributions to this study. We would also like to acknowledge all technicians in the laboratory for their excellent work during sample processing and analysis. We would like to acknowledge all participants from the marital screening program, who cooperated in this study and made it possible.

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