Original Paper

Premarital Screening Program for Hemoglobinopathies in Karbala, Iraq

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Abstract

Background: Premarital screening program for hemoglobinopathies started in Karbala governorate on June13, 2012.
Aim: To evaluate the premarital screening program in Karbala governorate.
Methods: A cross-sectional study by reviewing the records of five premarital clinics and Karbala hereditary blood disease center from first of March 2016 to first of October 2018. Total number of individuals screened for hemoglobinopathies were 52014 (26007 couples). Complete blood count (CBC) was done for all screened couples. If both man and woman have mean corpuscular volume (MCV) < 80 femto liter (fl) and/or mean corpuscular hemoglobin (MCH) < 27 picogram (pg) they should be referred to Karbala hereditary blood diseases center for further investigation of abnormal hemoglobin. Prevalence of abnormal hemoglobin calculated by Punnett Square equation and Hardy-Weinberg law.
Results: Number of individuals referred from peripheral premarital clinics to the center were 1780 (890 couples). Individuals included in the study were1560 (87.7%) from Karbala governorate, the rest with missing address or from other governorates were excluded. The most prevalent hemoglobinopathy among referred individuals was B-thalassemia 344(22.05%). Referred individuals with sickle hemoglobin were 51(3.26%). Prevalence of B-thalassemia and sickle hemoglobin in this study was 3.8% (95% confidence interval (CI) 3.64 – 3.96) and 0.56% (95% CI 0.5 – 0.62), respectively. High-risk marriage couples were 84 (3.2/1000).
Conclusion: The premarital screening for hemoglobinopathies program in Karbala governorate was able to detect many risky marriages although it still needs more improvement.
Keywords: Premarital screening, hemoglobinopathies, thalassemia, sickle cell anemia, Karbala

Introduction

Hemoglobinopathies are the most common single gene disorder worldwide [(1)]. More than 330000 affected infants are born globally annually [(2)], up to 90% of these births occur in low- or middle-income countries [(3)]. Hemoglobinopathies represent a global health burden with 3.4% deaths in children less than 5 years [(2)]. More than 1% of couples are at risk of having at least one affected child [(2)]. There are two types of hemoglobinopathies: thalassemias (decreased globin chain production) and hemoglobin structural abnormalities. Mixed types are common which have both features [(4)]. Advances in peptide sequencing, deoxyribonucleic acid (DNA) sequencing and mass spectrometry described more than 1000 abnormal hemoglobin mutations [(5)]. They are autosomal recessive mutations, and the homozygous, heterozygous, or compound heterozygous states result in disease with variable clinical severity [(6)]. World Health Organization (WHO) in 2006 urged member states to implement national programs for management and prevention of hemoglobinopathies to reduce morbidity and mortality of these diseases [(7,8)]. The goal of WHO is

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carrier identifying and genetic counseling with provision of effective measures to reduce these births. Iraq is a Middle Eastern country and its pivotal location in the center of the old world made it inhabited since ancient times. It is central to the thalassemia belt. In addition to the theory of malaria, all these facts made the country endemic with hemoglobinopathies. Consanguineous marriage is quite common in Iraq (47% - 60%) [(9)]. Iraqi ministry of health requested from all health directorates to mandate premarital screening for hemoglobinopathy to all couples attending court for official marriage certificate. Karbala governorate lies in middle of Iraq to the west of Euphrates. Karbala hereditary blood disease center was established in 2004 to manage and follow up the increasing numbers of patients with thalassemias and other hereditary blood diseases. The premarital screening program for hemoglobinopathies was started in Karbala governorate on June13, 2012. The aim of the program is reducing the birth of new cases of major hemoglobin disorder, while that of this study is to evaluate the premarital screening program in Karbala governorate.

Methods

A cross-sectional study reviewing the records of five premarital clinics and Karbala hereditary blood disease center from first of March 2016 to first of October 2018. When the premarital screening program for hemoglobinopathy in Karbala was started in 2012 one premarital clinic was established in Al-Husseiniya, Al-Hindiya and Ein-Altamr while two clinics were established in Al-Hussein and Obstetric hospitals in Karbala center district. Total number of individuals screened for hemoglobinopathies in this study was 52014 (26007 couples). Every couple applies for marriage certification are mandatorily required to visit their district hospital for premarital screening for hemoglobinopathy. 3 milliliter (ml) venous blood sample is collected from each individual in EDTA tube. CBC with differential is done within 4 hours using Sysmex XN-350 blood cell auto analyzer, which is calibrated according to manual user advice every 100 samples. Men blood sample tested first because carrier woman in our community may be considered flaw and decrease her chances of marriage. If the MCH was ≥27 pg. and the MCV was ≥80 fl., it would be safe completing marriage regarding hemoglobinopathy. If the man has MCV < 80 fl. and/or MCH < 27 pg. his partner woman blood sample should be tested for CBC. If the woman CBC was normal, the marriage is considered safe. If both man and woman have MCV < 80 fl. and/or MCH < 27 pg. they should be referred to Karbala hereditary blood diseases center to be tested for abnormal hemoglobin. In the center 5ml venous blood sample collected to test for hemoglobin electrophoresis and serum ferritin. Hemoglobin (Hb) electrophoresis is done with Bio-Rad D10 automated HPLC system, which is calibrated systematically with kit replacement as recommended by operator. Serum iron, total iron-binding capacity, blood film, sickling test and other necessary tests were done if needed to differentiate other causes of anemia. Abnormal hemoglobin suggestive diagnosis was according to the following criteria and clinical course:

B-thalassemia trait, Hb A2 ≥ 4% and Hb F 0.1– 5% [(10 p24)].

B-thalassemia intermedia, the onset of clinical presentation usually after the age of 2 years [(11 p122)], Hb level ∼ 7–10 g/dl, Hb F 10–50% may reach up 100% and Hb A2 > 4% [(10 p25).]

B-thalassemia major, the onset of clinical presentation usually before the age of 2 years [(11)], Hb level usually < 7 g/dl, Hb F up to 100% and Hb A2 is elevated [(10 p25)].

possible α – thalassemia traits, Hb F <1%, Hb A2 > 4% plus microcytic hypochromic RBC (confirmed by DNA Study) [(10 p24)].

Hb E constitutes of 25-30% of the total Hb. In Hb E carrier, while in homozygous state Hb E reaches up to 85-95% and Hb F 5-10% [(10 p21)].

Structural Hb Variants like Hb S, C and D this is achieved through HPLC, if the predominant Hb in the chromatogram was abnormal then it is a disease status. When S – Window < 50%, Hb A>50% for diagnosis of sickle cell trait and if S – Window > 50%, Hb A<50% for diagnosis of sickle cell disease [12 p2345].

SB-thalassemia+ is confirmed if at least 50% of the Hb Is Hb S, Hb A is present and the amount of Hb A2 is elevated (typically >3.5%). So far if S – Window < 50%, Hb A > 50% and Hb A2 > 3.5% for diagnosis of sickle thalassemia trait, compound heterozygosity [12 p2336,13]. These rules applied to other structural hemoglobinopathies like Hb D and C [13].

It was infeasible to do hemoglobin electrophoresis and sickling test to all screened couples. Screened couples were informed in details about steps done. Analysis of results and concluding the outcome of these marriages was clarified to couples in details. Certificate of hemoglobinopathy status supplied and genetic counselling was provided especially if there was risky marriage and they were sent back to the peripheral premarital clinics. Prevalence of hemoglobinopathy carriers in marriage certificate applicant was calculated. Punnett Square equation [14] was used to calculate the prevalence of hemoglobinopathies in screened individuals. Hardy Weinberg law is an alternative way to calculate allel frequencies from the genotype frequencies within a single generation [15]. The frequency of heterozygote carrier in population can be predicted by using allel frequencies [15].The estimated number of Karbala governorate population for 2018 was 1218732 [16] . The annual 2018 report of Karbala Hereditary Blood Diseases Center total number of registered homozygous thalassemia was (555) and sickle cell disease was (204) patients in Karbala governorate. Couple signed written consent before they were enrolled in this study assuring confidentiality of results for all participants.

Results

Couples in which both man and woman with MCV < 80 fl. and/or MCH < 27 pg. referred to Karbala hereditary blood disease center from peripheral clinics were 1780 individuals (890 couples). Individuals with missing addresses 43(2.4%) and from other governorates 177 (10%) were excluded. Individuals included in the study were1560 (87.7%) from Karbala governorate (table 1). Individuals from Karbala center district 983(63%), Al-Husseiniya 258(16%), Al-Hindiya 230(14%) and Ein-Altamr 89(5.7%) (table 2). Mean age± standard deviation (SD) of men 24(±6.1) year with minimum 14 and maximum 60 years while women have mean age±SD of 19.7(±4.6) year with minimum 13 and maximum 45 years. Out of the 1560 individuals referred to Karbala hereditary blood disease center 395(25.3%) have abnormal hemoglobin. The most prevalent abnormal hemoglobin in referred individuals was B-thalassemia 344(22.05%) which includes B-thalassemia minor 322(20.6%), sickle thalassemia 20(1.28%) and B-thalassemia intermedia 2(0.12%). Referred individuals with sickle hemoglobin were 51(3.26%) which includes sickle thalassemia 20(1.28%), sickle trait 27(1.73%) and sickle cell disease 4(0.25%). Hemoglobin HbAD 13(0.83%) and other rare hemoglobinopathies (table 3) and (figure 1). The highest proportion of B-thalassemia hemoglobin found in individuals referred from Al-Husseiniya (26%) followed by Karbala center district (22.1%), Al-Hindiya (19.5%) and Ein-Altamr (14.6%) (table 4, 5). The highest proportion of sickle hemoglobin (sickle cell disease, sickle trait and sickle-thalassemia) found in individuals referred from Ein-Altamr (14.6%), followed by Al-Husseiniya (4.2%), Karbala district (2.5%) and Al-Hindiya (0.86%) (table 4, 6). Calculating prevalence of B-thalassemia and sickle hemoglobin in this study using Punnett Square equation was 3.8% (95% CI 3.64 – 3.96) and 0.56% (95% CI 0.5 – 0.62), respectively. The predicted prevalence of B-

thalassemia and sickle hemoglobin for Karbala governorate using Hardy-Weinberg equation is 4.17% (95% CI 4.14 – 4.21) and 2.55% (95% CI 2.52 – 2.58), respectively. High-risk marriage couples in which both man and woman have abnormal hemoglobin and might lead to offspring with major hemoglobinopathies were 84 (3.2/1000) out of 26007 screened couples. Couples at risk having children with thalassemia and sickle disease were 70 (2.7/1000) and 13 (0.5/1000), respectively. They had proper genetic counselling and the decision to complete marriage was their full responsibility.

Table 1. geographic distribution of study subjects

		Frequency	Percent
City	Karbala	1560	87.7
	Hilla	47	2.6
	Baghdad	46	2.6
	Najaf	29	1.6
	Diwaniyah	19	1.1
	Basra	17	1.0
	Nasiriyah	7	0.4
	Kut	6	0.3
	Amara	2	0.1
	Mosul	1	0.1
	Samawah	1	0.1
	Diyala	1	0.1
	Erbil	1	0.1
No Address		43	2.4
Total		1780	100

Table 2. geographic distribution of study subjects within Karbala governorate

District	Frequency	Percent
Karbala center	983	63.0
Ein-Altamr	89	5.7
Al-Hindiya	230	14.7
Al-Husseiniya	258	16.5
Total	1560	100

Table 3. Abnormal hemoglobin among referred individuals

		Frequency	Abnormal Hb Percent	Total Percent
Abnormal Hb	B-thalassemia minor	322	81.5	20.6
	Sickle cell trait	27	6.83	1.73
	Sickle thalassemia trait	20	5.06	1.28
	HbAD	13	3.29	0.83
	HbAC	5	1.26	0.32
	Sickle cell disease	4	1.01	0.25
	B-thalassemia intermedia	2	0.5	0.12
	Alpha thalassemia	1	0.25	0.06
	HbE	1	0.25	0.06
	Total	395	100.0	25.3
Normal Hb		1165		74.7
Grand Total		1560		100

Table 4. Abnormal hemoglobin among referred individuals from peripheral clinics

District			Frequency	Abnormal Hb Percent	Total Percent
Karbala center	Abnormal Hb	B-thalassemia minor	205	85.06	20.8
		Sickle thalassemia trait	13	5.39	1.32
		Sickle cell trait	11	4.56	1.12
		HbAD	8	3.32	0.81
		HbAC	2	0.83	0.2
		Sickle cell disease	1	0.41	0.1
		B-thalassemia intermediae	1	0.41	0.1
		Total	241	100.0	24.5
	Normal Hb		742		75.5
	Grand Total		983		100
Ein-Altamr	Abnormal Hb	B-thalassemia minor	11	40.7	12.45
		Sickle cell trait	9	33.3	10.11
		Sickle thalassemia trait	2	7.4	2.24
		HbAD	2	7.4	2.24
		Sickle cell disease	2	7.4	2.24
		HbAC	1	3.7	1.12
		Total	27	100.0	30.33
	Normal Hb		62		69
	Grand Total		89		100
Al-Hindiya	Abnormal Hb	B-thalassemia minor	44	86.27	19.13
		Sickle cell trait	2	3.92	0.86
		HbAC	2	3.92	0.86
		Alpha thalassemia	1	1.96	0.43
		HbE	1	1.96	0.43
		Total	51	100.0	22.17
	Normal Hb		179		77.82
	Grand Total		230		100
Al-Husseiniya	Abnormal Hb	B-thalassemia minor	62	81.57	24.03
		Sickle thalassemia trait	5	6.57	1.93
		Sickle cell trait	5	6.57	1.93
		HbAD	3	3.94	1.16
		Sickle cell disease	1	1.31	0.38
		Total	76	100.0	29.5
	Normal Hb		182		70.5
	Grand Total		258		100

Table 5. B-Thalassemia among referred individuals from peripheral clinics

District	referred	B-thalassemia N (%)
Karbala center	983	219(22.2)
Ein-Altamr	89	13(14.6)
Al-Hindiya	230	45(19.5)
Al-Husseiniya	258	67(26)

Table 6. Sickle Hemoglobin among referred individuals from peripheral clinics

District	Referred	Sickle Hb N (%)
Karbala center	983	25(2.5)
Ein-Altamr	89	13(14.6)
Al-Hindiya	230	2(0.86)
Al-Husseiniya	258	11(4.2)

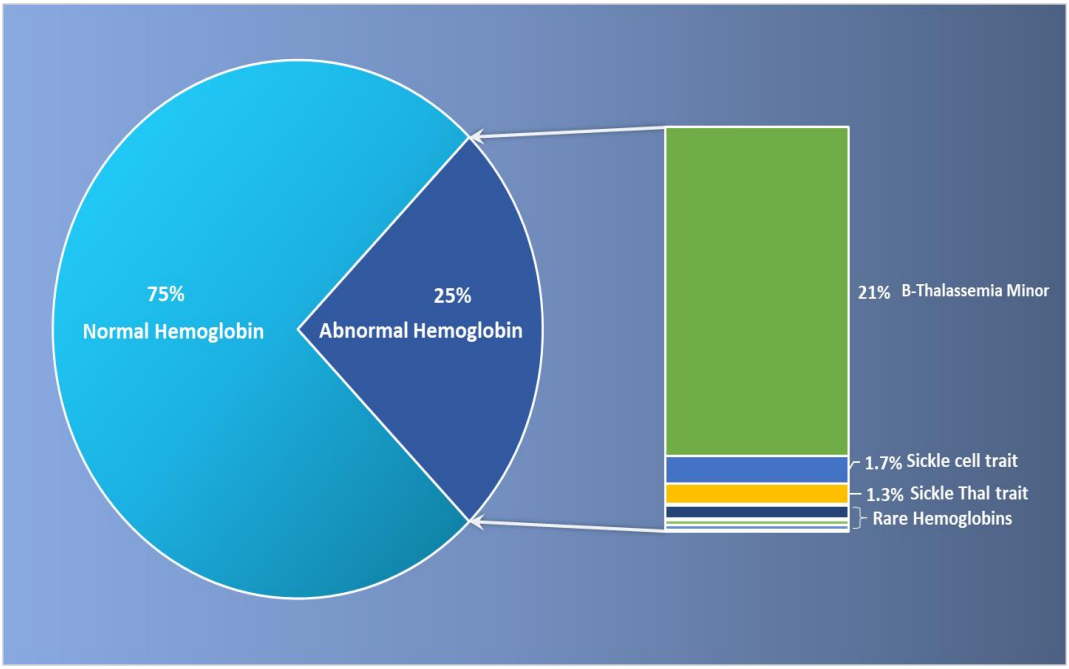

Figure 1. Abnormal hemoglobin among referred individuals

Discussion

Karbala hereditary blood disease center is the only center dealing with major hemoglobinopathy diseases in Karbala governorate. It is well known that one B-thalassemia major patient treatment and health services costs US$2500 to US$10500 annually, depending on her or his age and weight [17,18]. One can imagine the total burden of these diseases on the ministry of health budget. This fact emphasizes the importance of launching a well-established nationwide prevention program.

Prevalence of B-thalassemia heterozygote carrier in this study is (3.8%) which is comparable to other studies in Iraq. In Dohuk 3.7% [19], Baghdad 4.4% [20] and Basra 4.6% [18]. The neighboring countries may differ or agree with this study results depending on the extent of the disease prevalence in their localities. In Jordan 3.0%–3.5% (21–23), Saudi Arabia 3.0%–3.4% [24,25], Turkey average of 4.3% [26] and Iranian Average 3.6% [27]. Prevalence of B-thalassemia in this study 3.8% is slightly less than 4.1% predicted for Karbala governorate by Hardy Weinberg equation.

The prevalence of sickle cell hemoglobin in this study 0.56% is low compared to Douhuk 1.2% [19] and Basra 6.5% [18] in Iraq. Other studies from neighboring countries results was also higher than this study, in Jordan 1%–4.5% [23,28], Saudi Arabia national average of 5.7% [25,29] and Turkey 4.9% [30].

The highest proportion of sickle cell hemoglobin among couples referred from Ein-Altamr 14.6% compared to other Karbala governorate districts, which is expected, because this is a tribal district with very high rates of endogamy and the area is known for

sickle cell disease. The prevalence of sickle hemoglobin in this study 0.56% is less than 2.5% predicted for Karbala governorate by Hardy Weinberg equation. Sickle cell carrier in this study was underestimated because screening method using CBC only will miss significant number of sickle hemoglobin carriers. All couple screened with CBC should have sickling test and hemoglobin electrophoresis to detect sickle cell carrier but this was infeasible. This might be a weak point in the screening program. It requires both couples to have low MCV and low MCH in order to perform electrophoresis which may lead to escape of some serious hemoglobinopathies like HbS or HbC when coinherted with thalassemia.

High-risk marriages calculated in this study (3.2/1000) is less than that found in Dohuk (5/1000) [19], Basra (10/1000) [18], Iran (3.8/1000) [31] and turkey (4.4/1000) [32]. This could be due to reduced detection rate of sickle hemoglobin in this study and that Basra and Dohuk are endemic regions with sickle cell hemoglobin.

Conclusion

Prevalence of B-thalassemia carrier in this study was (3.8%). It was comparable to other studies in Iraq and neighboring countries. While the prevalence of sickle cell hemoglobin was obviously, low 0.56%, which is not representing the true figure of sickle cell carriers in Karbala governorate. Screening for sickle cell hemoglobin is advised for all couples attending the premarital screening program for hemoglobinopathies. Sickle cell carrier in this study was underestimated because screening method using CBC only will miss significant number of sickle hemoglobin carriers. This also led to lower high-risk marriages detected in the study. Correcting this issue is crucial to reduce these high-risk marriages.

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