Frequency of Haemoglobinopathies in Premarital Screening in Nineveh Province

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ABSTRACT

Background: Haemoglobinopathy is a large heterogeneous group of genetic abnormalities of haemoglobin. It is one of the most common inherited diseases worldwide.

Aim of this study: This study aimed to find the frequency of different types of Haemoglobinopathies in premarital couples in Nineveh province.

Subjects and Methods: In this cross-sectional study, the subjects were couples who go to the primary health care centers in Nineveh governorate for routine premarital investigations and the data were collected from the main premarital screening centers in Nineveh governorate.

Results: In this study, 1127 cases were included. 613 (54.4 %) were male, their ages range between (13-80 years), and 514 (45.6 %) were female, their ages range between (10-52 years). 47 cases were diagnosed as βeta-thalassaemia carriers with an overall frequency of 4.2 %. Ten cases had haemoglobin S (HbS) by the High Performance Liquid Chromatography (HPLC) (sickle cell trait in 9 cases and sickle β-thalassaemia in only one case); the overall frequency of sickle cell carrier state is 0.89 %. Eight cases with an overall frequency of 0.71 % were diagnosed as having other types of Haemoglobinopathies (haemoglobin D, haemoglobin E, haemoglobin H). Eighty one cases were diagnosed as having iron deficiency with an overall frequency of 7.2 %. By using the Hardy –Weinberg equation; we found the expected number of children born with homozygous βeta-thalassaemia would be (0.3/1000 from those born) and homozygous sickle cell disease would be (0.01/1000 from those born).

Conclusions: β-thalassaemia trait represented the most frequent Haemoglobinopathy in the region, Iron deficiency was significantly higher in females than in males, HPLC is a good technique for routine use and the expected number of children born with homozygous β-thalassaemia, sickle cell and others (HbD, HbE, HbH) were (0.3/1000, 0.01/1000 and 0.0085/1000 from those born) respectively.

Keywords: Haemoglobinopathy , Nineveh , frequency .

تواتر اعتلالات خضاب الدم في فحص ما قبل الزواج في محافظة نينوى

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الخلاصة

الخلفية: اعتلال الهيموغلوبين هو مجموعة كبيرة غير متجانسة من التشوهات الجينية للهيموغلوبين. إنه أحد أكثر الأمراض الوراثية شيوعًا فى جميع أنحاء العالم.

الهدف من هذه الدراسة: تهدف هذه الدراسة إلى معرفة مدى انتشار أنواع مختلفة من اعتلالات الهيموغلوبين لدى الأزواج قبل الزواج فى محافظة نينوى.

الموضوعات والطرق: فى هذه الدراسة المقطعية ، كان المفحوصون أزواجًا يذهبون إلى مراكز الرعاية الصحية الأولية فى محافظة نينوى لإجراء فحوصات روتينية قبل الزواج ، وتم جمع البيانات من مراكز فحص ما قبل الزواج الرئيسية فى محافظة نينوى.

النتائج: فى هذه الدراسة ، تم تضمين ١١٢٧ حالة. ٦١٣ (٥٤.٤٪) من الذكور ، تتراوح أعمارهم بين (١٣-٨٠ سنة) ، و ٥١٤ (٤٥.٦٪) من الإناث ، وتتراوح أعمارهم بين (١٠-٥٢ سنة). تم تشخيص ٤٧ حالة على أنها حاملات للبيتا ثلاسيميا بمعدل انتشار إجمالى قدره ٤.٢٪. عشر حالات كان بها الهيموغلوبين S (HbS) بواسطة الكروماتوجرافيا السائلة عالية الأداء) (HPLC) سمة الخلية المنجلية فى ٩ حالات والمرض المنجل / الثلاسيميا فى حالة واحدة فقط) ؛ معدل الانتشار الإجمالى لحالة حامل الخلايا المنجلية هو ٠.٨٩٪. تم تشخيص ثمانى حالات مع انتشار إجمالى بنسبة ٠.٧١ ٪ على أنها مصابة بأنواع أخرى من اعتلالات الهيموغلوبين (الهيموغلوبين D ، الهيموغلوبين E ، الهيموغلوبين .(H تم تشخيص إحدى وثمانون حالة على أنها مصابة بنقص الحديد مع انتشار إجمالى قدره ٧.٢٪. باستخدام معادلة هاردى وينبرغ ؛ وجدنا أن العدد المتوقع للأطفال المولودين بالثلاسيمية المتماثلة اللواقح سيكون (٠.٣ / ١٠٠٠ من المولودين) ومرض الخلايا المنجلية متماثلة اللواقح سيكون (٠.٠١ / ١٠٠٠ من المولودين).

الاستنتاجات: صفة الثلاسيميا بيتا تمثل اعتلال الهيموغلوبين الأكثر انتشاراً فى المنطقة ، وكان نقص الحديد أعلى بشكل ملحوظ فى الإناث منه عند الذكور ، ويعتبر HPLC تقنية جيدة للاستخدام الروتينى والعدد المتوقع للأطفال المولودين بالثلاسيميا بيتا متماثلة اللواقح والخلايا المنجلية و والبعض الآخر (HbD) ، HbE، (HbHكانت (٠.٣ / ١٠٠٠ ، ٠.٠١ / ١٠٠٠ و ٠.٠٠٨٥ / ١٠٠٠ من المواليد) على التوالى.

الكلمات المفتاحية : اعتلال الهيموغلوبين ، نينوى ، تواتر .

INTRODUCTION

Haemoglobinopathy is a large heterogeneous group of genetic abnormalities of haemoglobin. It is one of the most common inherited diseases worldwide.[1]

About 7% of the populations worldwide are carriers for trait genes of haemoglobin disorders, especially sickle cell disease and thalassaemia. This is according to the World Health Organization.[2]

Approximately 300 000–400 000 infants are identified every year to have Haemoglobinopathy, as indicated by global evaluations.[3] In the Arab countries, inherited blood diseases, specifically "thalassaemia and sickle cell disease" are very frequent and the affected children in these countries are in a big torture.[4] The extent of the problem can be attributed to two main points. The consanguineous marriage and the preference of having a large family size, so the number of affected children could be increased.[5]

“Haemoglobinopathy” is a broad term that includes many abnormal genetic conditions of haemoglobin. These consist of two main groups:

- "Thalassaemia syndromes"
- Abnormal structural haemoglobin variants.

Both are due to mutations and/or deletions in the alpha (α) or Beta (β) globin genes. When gene error affects haemoglobin (Hb) synthesis, it results in thalassaemia with normal haemoglobin structure, while abnormal haemoglobin variant results from abnormal Hb structure.[6,7]

AIMS OF THE STUDY

Are to detect the frequency of different types of Haemoglobinopathies in premarital couples in Nineveh province , to detect the frequency of carriers of β-thalassaemia and sickle cell.

MATERIALS AND METHODS

Subjects:

The couples who attend for routine premarital investigations in the primary health care centers in Nineveh province were enrolled in this cross sectional study.

There are 4 primary health care centers covering the governorate that offer premarital investigations, two centers in Mosul city where the blood is taken and the blood group, virology screen & automated blood count were done, but the High Performance Liquid Chromatography (HPLC) is performed in Ibn Al-Atheer teaching hospital whereas in Talafar and Al-Hamdania there is one center in each district where all these tests with HPLC were done in the same center.

1127 subjects were included. All of them were of Iraqis' nationality.

This is a cross sectional study which was conducted between the 15th of November 2019 and the 15th of May 2020.

In general, whole blood was taken from both premarital couples to perform blood group, automated Complete Blood Count (CBC) (using Swelab / Coulter counter) and Hb variant analysis by HPLC (using the variant Π Beta Thalassaemia Short Program from Bio-Rad laboratories-USA) and also for virology to exclude contagious diseases like hepatitis B, C, human immune deficiency virus (HIV) & VDRL testing for syphilis. However, in Talafar hospital and Al-Hamdania primary health care center, the Hb variant analysis was first performed only for the male and if there was an abnormality detected in his analysis then female would have Hb variant analysis too, this is due to social issues.

Serum ferritin was measured for cases with low Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH).

The interpretation was based on the percentages of HbA2 and HbF in cases of suspected thalassaemia traits, and on the presence of other peaks and windows of variant haemoglobins and their percentages, such as HbS, D and others.
β-thalassaemia trait was diagnosed when the subject have (Hb A2 >3.5%).

Other Hb varriants were recognized by the HPLC when other variant haemoglobins windows were detected like Hb D, E and S & the frequency of each Hb variant was measured.

When a subject had a low Hb, low MCV, low MCH, borderline HbA2, measure serum ferritin to detect any evidence of iron deficiency anaemia which affect HbA2 level and reassess Hb variant after iron therapy.[8-10]

MCV and MCH were considered low when MCV<76 fl and MCH <25 pg depending on the reference normal range of the kit used by these centers.

In some suspicious cases further tests were performed like the capillary electrophoresis. After that the affected couples were advised not to marry and were informed about the risks and the possibility of having babies with Haemoglobinopathies.

The statistical analysis of the data was done by using spss, and a p value of < 0.05 was considered as significant.

Ethical issues: the data in this study was attained in de-identified design. Subjects' names were not involved and the privacy of the participants were protected. Moral consent requirements were achieved by the study team.

Estimation of the expected numbers of births with Haemoglobinopathies: We used the (Hardy-Weinberg equation which is used in recessively inherited single gene disorders), and since in the current study we determined the frequency of Haemoglobinopathies in Nineveh, the expected births with homozygous and double heterozygous were calculated by using the mentioned equation.
The Hardy Weinberg equation is (p2+2pq+q2 =1).[11]

RESULTS

In this cross sectional study, 1127 cases were included who attended the four premarital primary health care centers in Nineveh province. 613 (54.4 %) were male, their ages range between (13-80 years), and 514 (45.6 %) were female, their ages range between (10-52 years).

Of these 1127 subjects 47 cases were diagnosed as β-thalassaemia carriers with an overall frequency of 4.2 %. The frequency of β-thalassaemia in Mosul city is 3.7 %, while in Al-Hamdania and Talafar are 9.8 % and 6.5 % respectively. Ten cases had HbS by the HPLC (sickle cell trait in 9 cases and sickle\β-thalassaemia in only one case), the overall frequency of sickle cell carrier state is 0.89 %.The frequency varied between 0.8 % in Mosul and 2.2 in Talafar, while it is 0 % in Al-Hamdania.

Eight cases with an overall frequency of 0.71 % were diagnosed as having other types of Haemoglobinopathies (HbD, HbE, HbH). It is 0.8 % in Mosul and 0 % in both Talafar and Al-Hamdania.

Eighty one cases were diagnosed as having iron deficiency with an overall frequency of 7.2 %. The iron deficiency frequency in Mosul is 7.8 %, while it is 2.4 % in Al-Hamdania and 2.2 % in Talafar, as shown in table 1 .

Table (1): The frequency & distribution of Haemoglobinopathies and iron deficiency in various regions of Nineveh province.

District		B-thalassemia trait		Sickle cell trait		Other hemoglobinopathies		Iron deficiency	
		M	F	M	F	M	F	M	F
Mosul	No.	24	13	3	5	6	2	8	70
	%	64.9 %	35.1%	37.5 %	62.5 %	75.0%	25.0%	10.3%	89.7%
	Total	37/994= **3.7%**		8/994 = **0.8%**		8/994 = **0.8%**		78/994 = **7.8%**	
Al-Hamdania	No.	4	-	- -		-	-	-	1
	%	100%	-	-	-	-	-	-	100.0%
	Total	4/41 = **9.8%**		0%		0%		1/41 = **2.4%**	
Talafar	No.	3	3	1	1	-	-	1	1
	%	50%	50%	50.0 %	50.0 %	-	-	50.0%	50.0%
	Total	6/92 =**6.5%**		2/92 = **2.2%**		0%		2/92 = **2.2%**	
Total	No.	31	16	4	6	6	2	9	72
	%	66.0 %	34.0%	40.0 %	60.0 %	75.0%	25.0%	11.1%	88.9%
For all	No.	**47**		**10**		**8**		**81**	
	%	47/1127=**4.2%**		10/1127=**0.89%**		8/1127=**0.71%**		81/1127=**7.2%**	

DISCUSSION

Nineveh is the second largest Iraqi province regarding population size which is approximately three and half million. It lies at the northwest of Iraq. It consists of diverse ethnic groups of population that include Arabs mainly, Kurdish, Turkmen and others. This diversity reflected by the presence of different types of Haemoglobinopathies. The premarital screening program is a way to detect carriers of Haemoglobinopathies and the couples at risk (couples who are both carriers and at risk of having diseased child if getting married), thus it is considered an effective way to reduce the frequency of Haemoglobinopathies mainly thalassaemia and SCD and decrease the burden of their consequences on population and the health care providers.

In this study, we depended on the premarital screening program in the province to find the frequency of different Haemoglobinopathies in the region using the HPLC technique and some other hematological and biochemical procedures. HPLC is a very useful and powerful technique; it quantifies HbA2, HbF and other Hb variants if present, all at the same technique. It can detect many Hb variants and β-thalassaemia trait; therefore, it is optimal for routine use in premarital screening program.

The frequency rate of **β-thalassaemia trait** in Nineveh governorate is 4.2% and it is consistent with the rates reported from other Iraqi's regions such as from the Iraqi's capital "Baghdad" in 1996.[12] 4.4%, Duhok governorate 4%.[13], from Basra in South of Iraq 4.6%.[14], while in Erbil , a province of Kurdistan /Iraq, the frequency of β-thalassaemia trait (6.94%).[15] The frequency of β-thalassaemia trait in the neighboring countries is variable. In Syria, carriers of β-thalassaemia trait are about 5%.[16] In Turkey, 4.3% as reported by the National Haemoglobinopathy Council, the highest frequency of β-thalassaemia trait (13.1%) was in Antalya.[17] In Jordan (in Badia region) the frequency is (3.04%).[18] While in Iran (20.66%) were carriers of β-thalassaemia.[19] & in Saudi Arabia (in Al-Hasa), 3.4 %.[20]

The frequency of **sickle cell trait** in Nineveh governorate is 0.89 %, in Iraq Sickle cell carrier rates range from 0 to 16.0%, and they aggregate in the south and the far north of Iraq.[21,22] In Basrah the overall frequency rate for the HbS is of 6.5%.[14]

In Erbil, Sickle cell trait (0.064%) [15]. In Duhok a study detected that the frequency of sickle cell trait was 1.2%.[13] Sickle cell disease is uncommon in Syria, and less than 1% of the populations are carriers.[23] In Iran (0.58%) were sickle traits.[19], whereas in Saudi Arabia, the frequency of sickle cell trait is (4.20%).[24]

In this study, it was found that the most common Haemoglobinopathies after thalassaemia and SCD are HbD then HbE and HbH. The frequency rate of HbD (0.44 %), HbE (0.18 %) and HbH (0.089 %). In Turkey the frequency rate of HbD was between 0.09% from a study in kocaeli.[25] and 0.28% from a study in Kahramanmaraş.[26] While in a study from Punjab, the frequency of HbD (1.09%) and HbE heterozygous (0.54%).[27] In Iran, the frequency of HbD variant is (0.84%) and HbE trait is (0.13%).[19] The frequency of Hb D-Iran was 0.23%, while that of Hb E was 1.56% in a study from India.[28] In Erbil, α-thalassaemia trait (0.032%).[15] In Iran, (0.29%) individuals with alpha thalassaemia variants (Hb-H disease/alpha trait).[19] Since this study depends on the premarital screening program in the region using HPLC for diagnosis of Haemoglobinopathies and since α-thalassaemia diagnosis is only by genetic study. Therefore, α-thalassaemia is not included in our study except for HbH disease where it could be diagnosed by HPLC.

HbD and HbE are clinically not that significant especially in heterozygous state, but it is important to detect them as a clinically significant condition may result if these are combined with β-thalassaemia trait.

Regarding the **iron deficiency** it was mentioned by (WHO) that the main cause of anaemia worldwide is iron deficiency.[29], especially in the developing countries.[30], and it is the most public and wide-ranging nutritional disorder globally.[31] In the developing countries, the high frequency of iron deficiency anaemia is caused by the ignorance for the diet quality due to the low socioeconomic status and nutritional factors like low iron containing diets.[32,33]

The overall frequency of iron deficiency in this studied group is 7.2% which was due to iron deficiency anaemia in the majority, but also a significant proportion was in iron deficiency state. Iron deficiency was mainly in females (72 females and only 9 males). The frequency of iron deficiency in a study from Duhok was 5.6%, and was also mainly in females.[13] From a study in Ramadi, frequency of iron deficiency anaemia (20.35%) and was also higher in females than in males.[34]

Depending on a study that took place in Turkey, the frequency of iron deficiency was 15.6% and of iron deficiency anaemia was 10.3%.[35] In Tabuk/Saudi Arabia the frequency was 12.5%.[36], while in a study in Saudi Arabia was 35.3%.[37] In Jordan, frequency rate of iron deficiency in females was (28.7%) and (11.1%) in males.[38] The frequency rate of iron deficiency from a study in Kermanshah/Iran was 23.7%.[39]

It was observed that the frequency of iron deficiency anaemia was also higher in females than in males that is consistent with our study in the following studies. [40-44,31,33,38] This may be attributed mainly to the periodic blood loss in females during menstruation.[45] and thus nutritional requirement is more for females in order to compensate the menstrual blood loss.[46-48]

Distribution of Haemoglobinopathies in Nineveh Province:

In this study the cases were in the majority taken from the premarital screening center in Mosul city, and fewer cases were taken from premarital screening centers in Al-Hamdania and in Talafar districts. This is because Mosul is the largest region in the province with the largest population size and the drainage of cases is the largest as it receives couples from all the surrounding villages and towns except Al-Hamdania and Talafar as these have their own centers.

Regarding β-thalassaemia trait it was found that the highest frequency rate of (9.8%) was in Al-Hamdania then (6.5%) in Talafar, while the least frequency rate of (3.7%) was in Mosul. This higher rate in Al-Hamdania and then in Talafar could be related to the diverse ethnicity between these regions and also could be related to social issues like consanguineous marriage which is with high rate in these areas.

The highest frequency rate of sickle cell trait was in Talafar (2.2%) and (0.8%) in Mosul, while it was (0%) in Al-Hamdania. This may be also contributed to the ethnicity and the consanguineous marriage.

Regarding the iron deficiency, the frequency rate was highest in Mosul (7.8%). Whereas in Al-Hamdania and Talafar were (2.4%) and (2.2%) respectively. This could be attributed to that the majority of cases with iron deficiency were females and as the screening program in Al-Hamdania and Talafar include males and only few females. So, the rate was apparently lower in these areas.

Estimation of Risk:

In this study, depending on the estimated frequency rates of β-thalassaemia trait, sickle cell trait and other Haemoglobinopathies and by using the Hardy –Weinberg equation; the expected birth rates of children born with major Haemoglobinopathy were as follow:

Homozygous β-thalassaemia would be (0.3/1000 from those born).

Sickle cell disease would be (0.01/1000 from those born).

Homozygous for other Haemoglobinopathies (HbH, HbE, HbD) would be (0.0085/1000 from those born) while in a study from Basrah, the anticipated rates of birth of homozygotes ß-thalassaemia were (0.52/1000) and of homozygotes HbS (1.05/1000).[14] ,and in Duhok the anticipated rate of homozygotes ß-thalassaemia was 0.697/1000.[13]

CONCLUSIONS

1. There are different types of Haemoglobinopathies in Nineveh province where β-thalassaemia trait represented the most frequent Haemoglobinopathy in the region followed by sickle cell trait, HbD, HbE then HbH disease.
2. Iron deficiency was also detected in this studied group which was significantly higher in females than in males.
3. HPLC is a good technique for routine use in premarital screening program that can detect most of the cases with Haemoglobinopathies except for few cases that may need further investigations.
4. The expected number of children born with homozygous β-thalassaemia, sickle cell and others (HbD, HbE, HbH) were (0.3/1000, 0.01/1000 and 0.0085/1000 from those born) respectively.

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