Prevalence of Minor β-thalassemia Based on RBC Indices among Final Suspected Individuals in Premarital Screening Program Referred to Genetic Laboratories

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Abstract

Introduction: The current study evaluated the value of red blood cell (RBC) indices and the corresponding cut-off points for β-thalassemia control programs in Iran.

Materials and Methods: 1,150 individuals (575 couples) with low RBC indices and normal hemoglobin A2 who had been referred to the Genetic Centre of Isfahan, were tested during pre-marital screening analyses, in the 2 year period, 2006-2008. β-thalassemia mutations were evaluated.

Results: β-thalassemia was identified in 67.8% of the cases with both mean corpuscular volume (MCV) less than 78fl and mean corpuscular hemoglobin (MCH) less than 26 pg. However, 4.1% of the individuals with 78≤ MCV≤ 80 tested positive for thalassemia. MCV showed a higher diagnostic reliability than MCH. However, the accompaniment of MCH<26 with MCV<78 increased the risk of thalassemia 35 times. A significant association was found between the prevalence of minor β-thalassemia and educational levels, race and familial relationships.

Conclusion: According to the results of this study, it seems that MCV=80fl can be used as a proper cut- off point for the screening of minor β-thalassemia. Although in the present study MCV was found to have a higher diagnostic reliability than MCH, MCH <26 along with MCV are very helpful indices for the counselor physician to estimate the risk of minor β-thalassemia more accurately.

Key words: Minor β-Thalassemia, Screening, MCH, MCV, Thalassemia Preventing Program,

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Introduction

The thalassemia prevention program in Iran is based upon premarital screening of β-thalassemic couples (carriers) in order to encourage them to participate in counseling and perform prenatal diagnosis (PND).(1) In this program, red cell indices are checked. If mean corpuscular hemoglobin (MCH) <27pg or mean corpuscular volume (MCV)<80fl (cut off values) are found in both couples, hemoglobin A2 concentrations are measured. If confirmed as characteristic for minor β-thalassemia (HbA2>3.5), the couples are referred for counseling. But, when both partners have red cell indices lower than the cut off values with an HbA2 concentration in the normal range, they are considered suspect. Then, they must undergo some additional stages including a course of iron therapy and a subsequent recheck of the indices with or

Table1: Prevalence of minor β-thalassemia in different races of the study population

Ethnic Groups	Thalassemia		
	Thalassemic	Non- Thalassemic	Total
Tork-Gashgaiee	383(46%)	449(54%)	832
Lor	204(79.6%)	52(20.4%)	256
Turk	24(57.1%)	18(42.9%)	42
Arab	13(81.2%)	3(18.8%)	16
Others	3	1	4
Total	620	530	1150

Table 2: Prevalence of β-thalassemia in terms of MCV

MCV	Thalassemia		
	Thalassemic	non- Thalassemic	Total
MCV> 80	0	10 (100%)	10
78≤ MCV≤ 80	8 (4.1%)	187 (95.9%)	195
MCV< 78	612 (64.7%)	333 (35.3%)	945
Total	620	530	1150

MCV: Mean Corposcular Volume

Table 3: Prevalence of minor β-thalassemia in terms of MCH

MCH	Thalassemia		
	Thalassemic	non- Thalassemic	Total
MCH> 27	1(4.5%)	21(95.5%)	22
26≤ MCH≤ 27	5(5%)	96(95%)	101
MCH< 26	614(59.8%)	413(40.2%)	1027
Total	620	530	1150

MCH: Mean Corpuscular Hemoglobin

Table 4: Prevalence of minor β-thalassemia in the study population in terms of MCV and MCH

DNA analysis	RBC indices				
	MCV	MCH			Total
		MCH≥ 27	27> MCH≥ 26	MCH< 26	
β- Thalassemia mutation	**MCV> 80**	0	0	0	0
	78≤ MCV≤ 80	1	2	5	8
	MCV< 78	0	3	609	612
	Total	1	5	614	620
Without β- thalassemia mutation	**MCV> 80**	0	6	4	10
	78≤ MCV≤ 80	16	51	120	187
	MCV< 78	5	39	289	333
	Total	21	96	413	530

RBC: Red Blood Cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin

without molecular studies.(2) The same process must be undergone when only one partner has fewer values of MCV and MCH and the other is thalassemia minor. These stages are time consuming, taking one month (in case of definite response to iron therapy) or more (with molecular studies) and also may cause high financial expenses for the families. Emotional stress and suspense during this period beside the financial expenses, often creates hardship for the couples and their families. In reference hematology books and also in literature, different cut off values have been mentioned for MCH and MCV which might be due to the difference in the characteristics of the study populations, such as age and race.(3-11)

Overall, in these studies, MCV cut- off values have been proposed from 75 to 80fL and MCH cut-off values suggested from 26 to 27. On the other hand, there is an on-going controversy regarding the choosing of MCV, MCH or both indices for β-thalassemia screenings.(10-12) Screening programs in Iran are based on MCV<80 and MCH<27 which may eliminate the undiagnosed cases, but increases the screening expenses and tensions for couples. Defining proper cut- off values in the screening program might prevent this unnecessary expenses and stresses.

This research was conducted to determine the prevalence of minor β-thalassemia based on RBC indices, and might be a beginning in defining proper cut- off values to screen minor β-thalassemia.

Materials and Methods

This cross- sectional study was conducted on finally diagnosed suspected individuals (with MCV<80 or

MCH<27, normal HbA2 concentrations, and no response to iron therapy) during national screening programs for β-thalassemia. These individuals had been referred for investigation of the related mutations to the Genetic Center of Isfahan (Iran) from Feb., 2006 to Jan., 2009. Blood samples were collected on 10% EDTA, and then PCR tests were performed on DNA using specific primers for common β-thalassemia mutations. If the case was negative for common mutations, the DNA was sequenced for other mutations. Finally, positive and negative cases were determined. The results of the tests were recorded on data sheets. In total, collected data included age, sex, race, educational levels, the familial relationships, MCV, MCH, and being thalassemic or not according to the PCR tests. The prevalence of minor β-thalassemia diagnosed by PCR test was calculated in terms of MCV and MCH values within four groups: 1) 78≤MCV≤80 and normal MCH, 2) 26≤MCH≤27 and normal MCV, 3) 78≤MCV≤80 and 26≤MCH≤27, 4) MCV<78 or MCH<26.

Data was analyzed using SPSS software and Chi square test.

Results

A total of 1,150 individuals (52% men and 48% women) referred to the "Isfahan Genetic Center" during the 2006- 2008 had been evaluated. In 53.9% of this population (53.2% of men and 54.7% of women), β-thalassemia mutations were found. In 46.7% of the cases, the educational levels were lower than high school (2.7% were uneducated), although 11.6% had a Bachelors or higher degree. The prevalence of minor β-thalassemia was significantly higher in individuals with less than a high school diploma (60.2% vs. 48.3%, p<0.001). 99.9% of the population was Moslem. The majorities (72.3%) were among the Turk-Ghashghaei people. The prevalence of minor β-thalassemia was highest among "Arab" and "Lor" ethnic groups, and "Turk-Ghashghaei" people had the least prevalence (Table- 1). The majority of referrals (56.8%) were unrelated to with their mates, and 28.2% had a first- degree familial relationship with their husband/wife. The prevalence of minor β-thalassemia was significantly higher among those who had a close familial relationship with their mate than those without any familial relationship (63% vs. 45.8%, p<0.001). Although 18% of this population had MCV values higher than 78fl, only 1.3% showed mutations related to thalassemia. None of the 10 individuals with MCV≥80fl were β-thalassemic, but in 4.1% of those with 78≤MCV<80 and in 64.7% with MCV<78 minor β-thalassemia was identified (Table- 2). 123 individuals (10.7%) had MCH ≥26pg (1.9%≥27pg). Of 22 individuals with MCH≥27, only one had β-thalassemia and 5% of those with 26≤MCH<27, and 59.8% of those with MCH<26 pg were found to have minor β-thalassemia (Table- 3). None of the statistics mentioned above were significantly different between men and women. Incidence risk of minor β-thalassemia in the case of MCH<26 (regardless of MCV values) was 30 times higher than whit MCH>26. For MCV<78, the incidence risk was 46 times higher than with MCV>78. In cases with MCH>27 and 26≤MCH<27, the possibility of minor β-thalassemia had no correlation with MCV indices. However, in cases with MCV<78 and MCH <26, the prevalence of β-thalassemia was 35 times greater compared with cases with MCH>26. (Table- 4).

Discussion

In the present study, we investigated the prevalence of minor β-thalassemia in terms of MCV and MCH values among finally suspected diagnosed individuals in the national screening programs for thalassemia referred to the Isfahan Genetic Center during the years 2006- 2008. No significant difference in the prevalence of minor β-thalassemia between men and women can be explained by the fact of autosomal recessive transmission of the trait. Thalassemia screening in Iran is mandatory before marriage, as well as "counseling program" for prenatal diagnosis if thalassemia minor is confirmed in a couple. The necessity of compulsory premarital screening programs is under debate. Some organizations argue that screening programs should be voluntary.

However, to encourage the general population to take part in these kinds of programs, advanced educational plans are required. In developing countries, because of poor social awareness regarding genetic diseases, genetically related marriages is a common problem.(13)

The significant relationship between the prevalence of thalassemia and educational levels in this population (which is statistically less prevalent in more educated people), might be due to more information about this trait and, therefore, the avoidance of marriage with relatives. Until effective educational campaigns can be set up to inform the general population about thalassemia, such as school educational programs and various media

advertisements, the premarital screening programs may remain mandatory.

A higher prevalence of minor beta thalassemia was expected within some ethnic groups in Iran. Fars and "Kohgiluye and Boyerahmad" provinces, which contain a high proportion of Lor and Turk-Ghashghaei ethnic populations are among the 10 provinces with the highest prevalence of β-thalassemia in Iran.(14-15) A significant increase in the prevalence among those who are relatives with their mates was also expected due to the hereditary nature of the trait.

Although the number of investigated cases in groups with "MCV>80 and MCH<27" and/or "MCV<80 and MCH>27" was not enough, the presence of one minor β-thalassemic patient among 22 individuals with MCH>27 and no β-thalassemia was found among 10 individuals with MCV>80 which might indicate the preference of the MCV index on MCH. In other words, if we used the criteria suggested by Pignatti (MCV< 78 and MCH<27as a cut off),(11) then, of the 17 cases with MCV>78 and MCH>27, only one error would be found (5.9%). But, when considering MCV<80 as the only criteria, no false negatives were found and, therefore, there would be no need to screen the 10 cases with MCV≥80 and MCH<27 (no case was found in this group) (Table- 4). Our results are in contrast with those of Karimi, et al,(12, 16) since we found that MCV is a more sensitive hematological index than MCH for predicting minor beta thalassemia. However, there are also other studies confirming our results, in which the emphasis is on MCV being more sensitive than MCH in this regard.(9,10,17,18) Some other studies even have declined to accept any of the indices as enough, and have suggested that RBC counts and RDW as more reliable indices, if concomitant with MCV and MCH (19, 20).

About these differences in literature, two facts must be considered: first, that, in Iran, national screening programs for β-thalassemia, no response to a complete course of iron therapy (60 mg ferrous sulfate, 3 times a day for 1-3 months) regardless of the prevalence of iron deficiency anemia in the community, rules out the possibility of iron deficiency. Second, as mentioned in Kiss's study, in a multicultural country, cultural characteristics of the ethnic groups in each region must be considered, as well as behavioral and food habits.(10)

A high prevalence of iron deficiency in a region can change the proper cut off values for the screening of minor β-thalassemia and, so, some researchers have even recommended MCV<76fl as a screening index in their region.(9) These facts might indicate some of the reasons for different results in the studies.

Although in some studies, the reliance on MCV as the only criteria has been considered enough.(17) Some other studies, have accepted MCH as the only index(12, 16) and a third group has accepted a combination of these two indices together (at least one, abnormal).(3-5,11) In our study, with values of MCH, no case of minor β-thalassemia was identified in MCV>80. According to our results, a higher accuracy was considered for MCV rather than MCH (if the cut- off value is considered 80fl). However, in cases with MCV<80 and MCH<26, the prevalence of β-thalassemia is 35 times more than when MCH is >26. Therefore, it seems more logical to consider both indices for thalassemia screening tests. In other words, if an individual has both MCV<78 and MCH<26, the chance of he/she being minor β-thalassemic is much higher than when both indices are over these values.

It must be considered that passing these stages is time consuming (1 to 3 months for iron therapy and then almost 2 months for molecular investigation of β-thalassemia mutations). It imposes a lot of emotional and financial stress on young couples in their premarital period. In some cases, when the couples cancel their marriage plans if PND is needed, they experience emotional turmoil during in this period. Informing the couples of the future possibilities is often useful. Although performing PND is now possible in our country, issues such as the risk of misdiagnosis, the possible problems of twin pregnancies, religious taboos, a 25% possibility of repeated major β-thalassemia in subsequent pregnancies, parental concerns about the consequences of a pregnancy or fear of an abortion, along with the direct and indirect expense of undergoing PND, are among the most important factors influencing decision making regarding marriage in minor couples. On the other hand, cultural problems (particularly for girls) in the case of dispensing of the marriage after a long engagement period may cause social problems. Being informed of the high or low probability of thalassemia can influence the behavior of the couple and their families during the engagement period and, therefore, reduce subsequent social problems.

Conclusion

Although this study did not aim to define a cut off point for MCV and MCH values in screening minor β-thalassemia, it seems that MCV<80fl is a proper

cut off value. However, an MCH value is very helpful in estimating the risk of minor β-thalassemia, particularly in cases with low MCV values. Low MCV values, when accompanied with MCH<26 pg, demonstrate a higher risk of β-thalassemia among couples.

Coauthor contributions

Alireza Moafi developed the concept, interpreted the data, wrote and revised the manuscript. Maryam Moamenzadeh and Mehrdad Zainalian performed clinical evaluations. Molecular diagnosis was done under the supervision of Sadeg Vailan. Zahra Nikyar collected the data. Soheila Rahgozar revised the manuscript and contributed for the interpretation of the data

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