Turkish Journal of Medical Sciences

Volume 42 | Number 2 Article 12

1-1-2012

Discrimination of beta-thalassemia minor and iron deficiency anemia by screening test for red blood cell indices

AZIZ BATEBI

ABOLGHASEM POURREZA

REZA ESMAILIAN

TÜBİTAK

Original Article

Turk J Med Sci
2012; 42 (2): 275-280
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0909-294

Discrimination of beta-thalassemia minor and iron deficiency anemia by screening test for red blood cell indices

Aziz BATEBI[1], Abolghasem POURREZA[2], Reza ESMAILIAN[3]

Aim: To assess the state of iron deficiency anemia and thalassemia minor among couples intending to marry at the Molla Hadi Sabzevari Health Clinic in Isfahan, Iran.

Materials and methods: Couples who intend to marry have to undergo these tests as part of a compulsory countrywide program. In this study, the red blood cell (RBC) indices among the study population were measured with an electronic cell counter (Sysmex K800). The survey involved the measurement of 2 RBC indices, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), and the results were compared with the England and Fraser, Shine and Lal, and Mentzer indices.

Results: The study population included 11,900 individuals, of whom 901 were recognized as having hypochromia (MCH < 32 pg/cell) and microcytosis (MCV < 80 fL). Of these 901 persons, 444 had developed beta-thalassemia, and 457 had iron deficiency anemia or were affected by other causes of microcytosis and hypochromia.

Conclusion: RBC indices and 3 other index components were examined with the screening test. The screening precision was based on the cut-off point of the hemoglobin A2 scale detected by column chromatography, or by DNA amplification by polymerase chain reaction. The findings reveal that the England and Fraser index, with a sensitivity level of 87.2%, is an acceptable discriminator of thalassemic and nonthalassemic patients. All indices functioned more accurately for men than for women.

Key words: Thalassemia minor, iron deficiency anemia, RBC indices, hemoglobinopathy

Introduction

Although thalassemic disorders are categorized as hemoglobinopathies, they result mainly from fluctuations of normal hemoglobin levels during life, being low or high, rather than from the production of abnormal hemoglobin (1,2).

Thalassemia is the most common type of hemoglobinopathy transmitted by heredity. These disorders are the result of the existence of an abnormal allele in one or more globin genes. The decrease or loss of an α or β chain, as 2 types of thalassemia, has unfavorable effects on the production and the survival of red blood cells (RBCs) and may cause a decrease in the concentration of the globin chain and of hemoglobin, resulting in microcytosis and hypochromia.

Thalassemia major is a worldwide disease, but it is more common in the Mediterranean region, the Middle East, the Asian subcontinent, and southeastern Asia, as well as southwestern Europe and central Africa.

Received: 19.09.2009 – Accepted: 20.01.2011

[1] Department of Health Education and Promotion, School of Public Health, Tehran Medical Sciences University, Tehran - IRAN
[2] Department of Health Economics and Management, School of Public Health, Tehran Medical Sciences University, Tehran - IRAN
[3] Molla Hadi Sabzevari Health Clinic, Isfahan Medical Sciences University, Isfahan - IRAN
Correspondence: *Abolghasem POURREZA, Department of Health Economics and Management, School of Public Health, Tehran Medical Sciences University, Tehran - IRAN*
E-mail: abolghasemp@yahoo.com

Iran suffers from a large number of thalassemia major patients; however, cases of α-thalassemia are very rare. The gene frequency of β-thalassemia, however, is high, and varies considerably from area to area, with the highest rate of more than 10% being found around the Caspian Sea and the Persian Gulf. The prevalence of the disease in other areas is between 4% and 8%. In Isfahan, the frequency is about 8% (3). There are approximately 15,000 patients registered as having thalassemia major in Iran, but it is estimated that the real number may be somewhat larger.

Researchers have paid much attention to differentiating thalassemia minor from the other types of hypochromic and microcytic anemia, because other types of hypochromic anemia are not transferred as a hereditary disorder. There is no legal prohibition against the marriage of thalassemic individuals in Iran; the only preventive application is informing new couples about the possible consequences of such marriages.

A definitive differential diagnosis between thalassemia minor and iron deficiency anemia (IDA) is based on the results of hemoglobin electrophoresis, serum iron levels, and ferritin calculation (4). A rather complex algorithm is used for the detection of thalassemia minor among volunteers who are intending to get married in Iran. In this process, detection of hypochromia and microcytosis by the measurement of RBC indices is a preliminary step. The Coulter counter is used for the rapid assessment and measurement of RBC indices (5).

Thalassemia major, like other recessive illnesses, can be eliminated if the carriers of the disease are fully detected and treated (6). Iran has pursued such an objective since 1998 among volunteers applying for a marriage license.

Governmental and private laboratories equipped to screen for thalassemia have been recruited into an accredited national professional laboratory network, supervised by a national reference laboratory and directorate for laboratory affairs. There are corresponding structures at the provincial level. Laboratory staff members follow national screening protocols based on international guidelines (6). The present study was carried out in 2007 at the Molla Hadi Sabzevari Health Center in Isfahan, as a provincial section of the entire program.

Materials and methods

Sample of the study

The purpose of the study was discrimination between beta-thalassemia minor (BTM) and IDA among couples intending to marry that were referred to the Molla Hadi Sabzevari Clinic in Isfahan, Iran, in 2007. Of 11,000 individuals (i.e. 5500 couples), 901 persons (440 females and 461 males) were detected as having hypochromia and microcytosis in their RBCs, as defined in the study proposal. Patients were selected according to the manual of the program.

Methods of diagnosis

For the detection of couples with hypochromia and microcytosis in their RBCs, 3 well-known methods of discrimination between BTM and IDA were employed. England and Fraser in March of 1973, Mentzer in April of 1973, and Shine and Lal in March of 1977 (7-9) proposed 3 separate indices for improving the method of diagnosis of microcytic and hypochromic anemia. These diagnoses are based primarily on RBC indices obtained via the Coulter counter analysis.

1. The England and Fraser index is reflected by the following equation:

(MCV – RBC + (5Hb) + k = ?), where MCV is mean corpuscular volume and Hb is hemoglobin. Thalassemia minor is indicated if the result is less than 0, while values of 0 or greater demonstrate nonthalassemic anemia (7).

2. The Shine and Lal index is obtained by multiplying the MCV by the RBC value. If the result is less than 1530, thalassemia minor is recognized, but a larger value indicates nonthalassemic anemia (8).

3. The Mentzer index is obtained when the MCV is divided by the mean corpuscular hemoglobin (MCH) value. If the result is equal to or greater than 13, thalassemia minor is recognized; otherwise, nonthalassemic anemia is indicated (9).

Methods of analysis

The 3 indices mentioned above, with the main index of the MCV and/or MCH measurements, were compared within the study population in 2007. The results of all of the indices were compared for the amounts of hemoglobin A2 (HbA2). The latter

was quantified by Hb electrophoresis following column chromatography, and the polymerase chain reaction (PCR) was used when the results of the Hb electrophoresis were inconclusive (10). For the final conclusion, the specificity and sensitivity of each index was determined. To be more precise in the calculations, the kappa coefficient was employed. The main purpose of these approaches was to set the priority of one of these methods against the rest, to be able to utilize these simple indices in the mass screening process, and to carry out a routine clinical analysis.

Couples intending to get married were examined at different stages (6). In all of the stages, the analysis of RBC values was conducted with a Sysmex K800 Coulter cell counter. Thalassemia minor was recognized by the microcytosis and hypochromia of the RBCs using a peripheral blood smear. The country's program for thalassemia control indicates that the cut-off point of the MCV for microcytosis is less than 80 fL, and that the MCH for hypochromia is less than 27 pg/cell. HbA2 was measured by column chromatography using the Helena SAS-MX alkaline gel Hb kit (Helena Laboratories, Tyne and Wear, UK).

Results

The study population included 440 women and 461 men. The final analysis showed that 444 persons had BTM and the remaining 457 persons had nonthalassemic causes of hypochromia and microcytosis such as IDA, chronic diseases, and rare causes such as lead poisoning and sideroblastic anemia. However, the country's program has no protocol for definite diagnosis of the sicknesses of the nonthalassemic group. Further studies are necessary to identify these conditions in detail (11).

Of the 444 patients who were recognized as having thalassemia minor, 273 were male and 171 female, and the nonthalassemic persons included 188 males and 261 females. The different sex composition of these 2 groups could be attributed to a higher prevalence of IDA among women in Isfahan. Table 1 demonstrates the statistical characteristics of the 2 groups of the study.

According to Table 1, while the MCV in thalassemic patients was in the range of 50.6-78.8 fL, with an average of 66.4 fL, the corresponding values for nonthalassemics were 67.3-84.5 fL, with an

Table 1. RBC indices among marriage applicants with definite diagnosis of BTM and nonthalassemic anemia referred to the Molla Hadi Health Center in Isfahan, 2007.

Standard deviation	Mean	Maximum	Minimum	Type of anemia	Rate index
6.47	66.4	78.8	50.6	Thalassemia minor	MCV (fL)
4.49	79.0	84.5	67.3	Nonthalassemic	
1.77	4/20	1/25	15.3	Thalassemia minor	MCH (pg/cell)
1.84	25.1	26.8	19.9	Nonthalassemic	
1.11	12.6	15.5	10.5	Thalassemia minor	Hb (g/dL)
2.29	13.0	17.2	8.9	Nonthalassemic	
0.613	6.16	7.59	4.84	Thalassemia minor	RBC (n × 106/μL)
0.57	5.32	6.47	4.26	Nonthalassemic	
2.02	10.98	15.48	7.12	Thalassemia minor	Mentzer; ≥13 = thalassemia minor
1.789	15.03	19.11	11.59	Nonthalassemic	
232.87	1359.80	1972.86	774.18	Thalassemia minor	Shine & Lal; <1530 = thalassemia minor
231.77	1988.91	2222.35	1372.92	Nonthalassemic	
8.19	11.05	6.06	27.49	Thalassemia minor	England & Fraser; <0 = thalassemia minor
9.90	12.49	14.34	20.97	Nonthalassemic	

Formulations and cut-off values: England and Fraser index: (MCV – RBC + (5Hb) + k = ?), (<0 = BTM, 0≥ = IDA).
Shine and Lal index: (multiplying MCV by RBC), (<1530 = BTM, ≥1530 = IDA).
Mentzer index: (MCV divided by MCH), (≥13 = BTM, <13 = IDA).

average of 79 fL. The significant difference ($P < 0.001$) between these values indicates that, in each person whose MCV is lower than the arbitrary microcytic cut-off point, the type of anemia, either thalassemic or nonthalassemic, could be recognized easily. This is why, in some texts, a MCV of less than 60 fL and a MCH value of less than 20 pg/cell are considered as indicators of a very low probability of having IDA and a higher probability of thalassemia minor (12).

The Mann-Whitney test showed a significant difference between all of the index distributions in the thalassemic and nonthalassemic groups ($P < 0.001$). Table 2 presents a summary of the sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively) of indices regarding sex, and thalassemia minor among all of the patients with hypochromia and microcytosis.

As indicated in Table 2, none of the indices studied demonstrated 100% precision in the recognition of thalassemia minor. In this study, the England and Fraser and the MCV indices demonstrated the highest and lowest sensitivity, respectively, and determined 87.2% and 81.3% of thalassemia minor. The highest and lowest indices calculated were from the Shine and Lal and the England and Fraser indices, which correctly recognized the nonthalassemic cases at 90.6% and 62.9%, respectively. In addition, Table 2 shows the highest and lowest PPV belonging to the Shine and Lal index with 89.6% and the England and Fraser index with 69.5%, respectively.

The Mentzer and MCV indices demonstrated the highest and lowest NPV, respectively, with 86.5% and 81.8%. The highest and lowest kappa agreement coefficients were those obtained with the Shine and Lal index, at 73.8%, and the England and Fraser index, at 49.8%, respectively. However, the kappa agreement coefficient of the MCV index was 0.629. The difference between the results of all of the indices mentioned above and the gold standard (HbA2) was statistically significant ($P < 0.001$).

The highest prevalence of IDA, and its coincidence with thalassemia minor among women, is regarded as one of the main reasons for a reduction in the accuracy of these indices in the recognition of thalassemia minor (13).

Sensitivity, PPV, and NPV were considerably higher among men compared to women. The specificity of all indices (except England and Fraser) was also higher among men than among women (14,15). However, the Shine and Lal NPV index was found to be somewhat higher among women than men (women: 85.7, men: 83.4) (16).

Table 2. Comparison of binary classification tests in the diagnosis of thalassemia minor and other causes of hypochromic microcytic anemia.

Index	Group	MCV	Mentzer	Shine & Lal	England & Fraser
Sensitivity	Men	88.6	94.5	86.4	96
	Women	69.6	73.1	77.8	73.1
	Total	81.3	86.3	83.1	87.2
Specificity	Men	95.8	93.7	98.9	54.2
	Women	73.4	79.2	84.8	68.8
	Total	81.7	85.4	90.6	62.9
Positive predictive value (PPV)	Men	94.9	95.9	99.2	75.3
	Women	62.6	69	76.4	59.8
	Total	81.1	85.1	89.6	69.5
Negative predictive value (NPV)	Men	85	92.2	83.4	90.3
	Women	79.2	82.2	85.7	96
	Total	94.9	92.2	86.4	73.1
Kappa agreement coefficient	Men	0.805	0.884	0.83	0.536
	Women	0.423	0.517	0.623	0.402
	P-value	<0.001	<0.001	<0.001	<0.001

Conclusion

IDA and BTM are recognized as the most important causes of hypochromia and microcytosis. In order to avoid much more expensive, time-consuming, and complicated procedures for discrimination between these disorders, researchers attempt to employ either RBC indices such as MCV, MCH, and red blood cell distribution width (RDW), or formulas derived from these indices. This process helps to select appropriate individuals for more detailed examination.

In the 1970s, to improve the quality of diagnosis and differentiation between thalassemia and other causes of microcytosis and hypochromia, a series of new indices were proposed by such investigators as England and Fraser (7), Shine and Lal (8), and Mentzer (9).

The MCV, RBC, and Hb concentrations were used to determine patients with microcytosis and for discrimination between IDA and thalassemia by England and Fraser. This approach worked properly and precisely. All of the cases, except for 1 out of 72, were easily recognized. The function took the following form: DF' = MCV – RBC – (5 × Hb) – 3.4. A positive value indicated iron deficiency and a negative value indicated thalassemia minor. The authors noted that this function is not applicable during pregnancy (7).

Another study, which was held in Turkey by Demir et al. in December 2002, showed that discrimination indices (such as those of Mentzer, England and Fraser, Srivastava, Green and King, and Shine and Lal), RBC count, RDW, and RDW index showed a sensitivity and specificity of 100%. Youden's indices of RBC count and RDW were the highest, with values of 82% and 80%, respectively. Of the patients, 90% and 92% were correctly identified with the RBC and RDW indices, respectively. It was also demonstrated that the RBC count and RDW index are the most reliable discrimination indices for differentiation between BTM and IDA (17).

In a study by Madan et al. in Delhi in 1999, a MCV below 80 fL and a MCH value below 27 pg were found to be very sensitive markers in the detection of BTM, even in the presence of iron deficiency ($P < 0.0001$) (18).

In the present study, the above indices were compared with ordinary RBC index calculations, including MCH and MCV. RBC index calculation is a routine method and a primary test of preliminary diagnosis of hypochromia and microcytosis, which is now practiced officially for marriage license applicants. As pointed out above, none of the indices had 100% precision in the recognition of thalassemia minor. Nevertheless, they do have a rather high precision and can help in thalassemia screening. Meanwhile, the England and Fraser index, with regard to its higher sensitivity (87.2%), seems to be an appropriate index for screening. The Shine and Lal index, with a coefficient of 0.738, has the highest correlation with final definite diagnosis tests. Therefore, it can be a useful index for physicians in their diagnoses. On the whole, it seems that the above indices that include several factors as catalysts may be less erroneous than MCV or MCH alone, although the England and Fraser index has a lower precision in comparison with MCV. The most important point in the analysis of these indices is their different precision for men and women. The results of these indices seem more reliable for men than for women.

This could be the result of the higher prevalence of IDA among women than men. Since the coincidence of IDA and thalassemia minor can cause a misdiagnosis by these indices, it is suggested that physicians should not rely entirely on the results of these indices when they face women who are suspected to have IDA. By contrast, these indices are highly reliable in communities with a low prevalence of IDA and among men.

Acknowledgments

We are grateful to our colleagues who contributed as consultants to the present study: Hodjat Zeraati, PhD; Fereshteh Majlessi, MD; and Poupak Derakhshandeh, PhD. We also thank the physicians and personnel of Molla Hadi Sabzevari Thalassemia and Genetic Center at the Isfahan Medical Sciences University for their cooperation, and Mrs Zohre Rahimi for her help at all stages of the study.

References

1. Cooly TB, Lee PA. Series of cases of splenomegaly in children with anemia and peculiar bone changes. Trans Am Pediatr Soc 1925; 37: 29-30.
2. Hoffman R, Benz EJ, Shattil S, Furie B, Cohen H. Hematology: basic principles and practice. 4th ed. Philadelphia (PA): Churchill Livingstone; 2005. p.557-82.
3. Habibzadeh F, Yadollahie M, Merat A, Haghshenas M. Thalassemia in Iran; an overview. Arch Irn Med 1998; 1: 27-33.
4. Goddard AF, McIntyre AS, Scott BB. Guidelines for the management of iron deficiency anaemia. Gut 2000; 46: 141-5.
5. Coulter counter. In: World of microbiology and immunology; 2003. Available from: URL: www.encyclopedia.com/doc/1G2-3409800150.html.
6. Samavat A, Modell B. Iranian national thalassaemia screening programme. BMJ 2004; 329: 1134-7.
7. England JM, Fraser PM. Differentiation of iron deficiency from thalassemia trait by routine blood count. Lancet 1973; 1: 449-52.
8. Shine I, Lal SA. A strategy to detect beta-thalassaemia minor. Lancet 1973; 1: 692-4.
9. Mentzer WC. Differentiation of iron deficiency from thalassemia trait. Lancet 1973; 1: 882-4.
10. Old JM. Screening and genetic diagnosis of haemoglobin disorders. *Blood Reviews* 2003; 17: 43-53.
11. Thomas C, Thomas L. Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency. Clinical Chemistry 2002; 48: 1066-76.
12. Milunsky A. Genetic disorders and the fetus: diagnosis, prevention, and treatment. 4th ed. Baltimore (MD): Johns Hopkins University Press; 1998. p.673.
13. Harrington AM, Ward PCJ, Kroft SH. Iron deficiency anemia, β-thalassemia minor, and anemia of chronic disease. Am J Clin Pathol 2008; 129: 466-71.
14. Beyan B, Kaptan K, Ifran A. Predictive value of discrimination indices in differential diagnosis of iron deficiency anemia and beta-thalassemia trait. European Journal of Haematology 2007; 78: 524-6.
15. Urrechagra E. Red blood cell microcytosis and hypochromia in the differential diagnosis of iron deficiency and beta-thalassaemia trait. International Journal of Laboratory Hematology 2009; 31: 528-34.
16. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pediatrics International 2002; 44: 612-6.
17. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. J Trop Pediatr 2004; 50: 369-71.
18. Madan N, Beachler L, Konstantinopoulos P, Worley S, Sun Z, Latson LA. Red cell indices and discriminant functions in the detection of beta-thalassaemia trait in a population with high prevalence of iron deficiency anaemia. Pediatr Cardiol 2010; 31: 1203-8.