# The Effect of Iron Deficiency Anemia ( IDA) on the HbA2 Level and Comparison of Hematologic Values Between IDA and Thalassemia Minor

**M. Reza KERAMATI*, N. Tayyebi MAYBODI****

* Mashhad University of Medical Science, Departement of Hematopathology, Neonatal Research Center
* Mashhad University of Medical Science, Departement of Pathology, IRAN

**ABSTRACT**

The most common hypochrom microcytic anemia are iron deficiency anemia (IDA) and thalassemia minor (TM). The results of some studies have shown that IDA can cause misdiagnosis of heterozygote β-thalassemia due to decrease in HbA2 level. Our aim in this study was evaluating the effect of IDA on HbA2 levels; Furthermore hematologic values in CBC of these two diseases will be compared.

In this study 291 individuals including normal control group, heterozygote α and β-thalassemia minor, IDA and coincident β-thalassemia and IDA patients were under investigation. CBC, serum ferritin, iron, and TIBC levels and hemoglobin electrophoresis in alkaline PH was managed for every subjects. They were then put into groups according to diagnostic criteria and were analyzed applying SPSS software (version 11.5) and statistical tests especially t- test.

HbA2 levels were 2.9%±0.4 in normal group, 2.7%±0.6 in IDA patients, 5.6%±0.9 in β-thalassemia minor, 4.7%±1 in coincident IDA and β-thalassemia minor. Above mentioned significant differences in HbA2 values are between normal and IDA individuals, also between β-thalassemia minor and coincident β-thalassemia and IDA patients. RBC counts, Hb, Hct, MCH, MCHC values were significantly higher in b- thalassemia minor comparing with IDA patients but MCV showed no significant difference in these two groups. RDW was increased in both, but it was higher in IDA. IDA can cause a decrease in HbA2 level. This point sometimes leads misdiagnosis particularly in coincident IDA and β-thalassemia minor. Therefore in suspicious cases of β-thalassemia trait in IDA background, it is better to do hemoglobin electrophoresis after treatment of IDA.

**Key Words:** HbA2, Thalassemia minor, Anemia, Hemoglobin electrophoresis, Iron deficiency

## ÖZET

**Demir Eksikliği Anemisinin HbA2 Seviyesine Etkisi ve Demir Eksikliği Anemisi ile Talassemi Minörün Hematolojik Parametrelerinin Karşılaştırılması**

En sık görülen hipokrom mikrositer anemiler, demir eksikliği anemisi (DEA) ve talassemi minördür (TM). Bazı çalışmalar DEA'nin HbA2 seviyesinde azalma olması nedeniyle heterozigot ß-talassemi ile karışabildiğini göstermiştir. Amacımız, DEA'nin HbA2 seviyesi üzerine etkisini değerlendirmek ve bu iki hastalıktaki kan parametrelerini, kan sayımlarını karşılaştırmaktır.

Bu çalışmaya normal kontrol grubu, heterozigot a ve ß-talassemi minör, DEA ve DEA ile ß-talassemisi birlikte olan 291 kişi alınmıştır. Tam kan sayımları, serum ferritin, demir düzeyleri, demir bağlama kapasiteleri ve alkalen pH'da Hb elektroforezleri çalışılmıştır. Bu değerler tanılara göre gruplanmış SPSS programında (11.5 versiyonu) t-testi kullanılarak karşılaştırılmıştır.

HbA2 düzeyleri normal grupta %2.9±0.4, DEA grubunda %2.7±0.6, ß-talassemi minör grubunda %5.6±0.9, DEA ve ß-talassemi birlikte olan grupta %4.7±1.0 bulunmuştur. Normal grupla kıyaslandığında HbA2 düzeylerinde bütün gruplarda istatistiksel anlamlı fark bulunmuştur.

ß-talassemi minör ile DEA karşılaştırıldığında MCV dışında RBC, Hb, Hct, MCH, MCHC değerleri arasında istatistiksel anlamlı fark izlendi. RDW herikisinde de artmıştı, ancak DEA'nde daha yüksekti.

DEA, HbA2 seviyesinde azalmaya neden olabilir. Bu durum, özellikle DEA ve ß-talassemi minör birlikteliğinde yanlış tanılara neden olabilir. Bu nedenle, DEA zemininde ß-talassemi trait varlığında DEA tedavisine başlamadan önce Hb elektroforezi ile tanının netleştirilmesi önerilmektedir.



**Anahtar Kelimeler:** HbA2, Talassemi minör, Anemi, Hb elektroforezi, Demir eksikliği


## INTRODUCTION

The most prevalent hypochrom microcytic anemia are iron deficiency and β-thalassemia trait (1,2). Iron deficiency anemia (IDA) and iron deficiency (ID) are common in Iran (3, 4). β-thalassemia is common in some particular zones such as Mediterranean area. There are exceeding 25000 cases of thalassemia major in Iran (5).

In some areas of Iran β-thalassemia prevalence is 3-4% (6,7). It is therefore important to have a laboratory diagnosis as there is a particular increasing need for prenatal detection of disorders with use in globin chain and verifying hemoglobin subgroups (8,9). Diagnosis β-thalassemia trait is being practiced by detecting increasing HbA2 level up to 3.5% (9,10,11).

Decreased amount of hemoglobin in IDA may reduce percentage of hemoglobin subgroups including HbA2. Therefore IDA is a potential diagnostic interference in these tests(11). Incorrect diagnosis can cause mistreatments and lead to chronic results especially for the offspring's of parents with β-thalassemia minor.(8) some specialists recommended rechecking HbA2 level succeeding IDA treatment in case of simultaneous β-thalassemia minor and IDA(11). So our initial aim of this study is evaluating the effect of IDA and iron deficiency on HbA2 levels.

Furthermore existing specifications in complete blood count (CBC) of individuals with IDA will be compared with thalassemia trait.

## MATERIALS and METHODS

291 individuals including normal control group from hematology and marriage consult clinics were grouped to be assayed for IDA or thalassemia minor in this study. Blood sample were collected from all patients in tubes containing, ETDA anticoagulant for CBC and hemoglobin electrophoresis and samples without anticoagulant to measure serum ferritin, Iron and TIBC (Transferritin Iron Binding Capacity). All samples were collected from fasting patients under standard conditions. CBC were analyzed by cell counter (Kx-21 sysmex) within 2 hours of sampling. This instrument was calibrated with reference methods and had a regular quality control program.

Hemoglobin electrophoresis was done with use of cellulose acetate gel in alkaline PH. Samples containing borderline HbA2 levels (3.4-3.6%), the values were rechecked by exchange cation chromatog-

raphy (Helena kit, France). $HbA_2 \geq 3/5\%$ was proved to be increased.

Serum iron, TIBC and ferritin were measured within 24 hours from sampling. Serum ferritin was measured applying radio immunoassay method (kawoshyar kit, Iran)

Ferritin was proved to be reduced less than 20 mg/L. Transferrin saturation for all patients were calculated.

Subjects without anemia or with hypochrom microcytic anemia and ferritin < 20 mg/L or transferrin saturation < 15% and $HbA_2$ < 3.5% were considered to have ID or IDA respectively Individuals with hypochrom microcytic anemia, $HbA_2 \geq 3.5\%$ and normal serum ferritin and transferrin saturation were diagnosed ß-thalassemia minor.

Patients with $HbA_2 \geq 3.5\%$ coincident with IDA considered having IDA plus β-thalassemia minor. For the patients who had normal hemoglobin electrophoresis, serum ferritin and transferring saturation and CBC featured likely as thalassemia minor they were diagnosed as α-thalassemia. Finally subjects with no particular disease and having normal hemoglobin electrophoresis, ferritin, iron, TIBC and CBC were considered as normal group (11, 12).

Variables were analyzed by SPSS (version 11.5) statistical software. Initial data summarized as means and standard deviation for continuous variables. Data were continuous with normal distribution. We used independent sample T test procedure and compare means for two groups of independent cases (Table 1). p value <0.05 was considered significant difference.

## RESULTS

From 291 individuals 222 cases were adults (>18 years), 69 were 1 to 17 years. Age range was 1 to 89 years with average 25.1 (±16.4). In adult group average years was 32.7(±13.9). From all subjects 59 cases were normal (30 male, 29 female), 17 with α-thalassemia minor (14 male, 3 female), 150 with b- thalassemia minor (64 male, 86 female), 55 with IDA (19 males 36 female), and 10 (3 male, 7 female) with coincident IDA and β-thalassemia minor.

$HbA_2$ levels of different groups in this study are showed in table (1). As it is proved in the table, mean $HbA_2$ was 2.9 (±0.4%) in normal groups, and %2.7 (±0.6) in IDA. This difference was significant.

Mean $HbA_2$ in β-thalassemia minor was 5.6% (±0.9) and in coincident ß-thalassemia and ID was 4.7 %( ±1).This difference was significant (table 1). Mean $HbA_2$ in patients with α-thalassemia was 3.1 %( ±1.3) which wasn't a significant difference comparing with normal group.

Leukocyte counts, had no significant difference in normal, in compare to IDA group, but there was a significant increase in leukocyte counts in α- and β-thalassemia minor corresponding normal and IDA patients.

Red blood cells (RBC), Hemoglobin (Hb), Hematocrit (Hct) in normal group had significant increase in male companying with female. It was observed a significant difference in RBC counts, RBC indices, Hb and Hct In both sexes, in α- and β-thalassemia in comparing with normal group.

There was also significant decrease in RBC, Hb, Hct, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) in IDA comparing with α- and β-thalassemia, but difference in MCV was not significant.

RBC counts had not significant difference in normal group comparing with IDA.

RDW showed significant increase in patients with heterozygote thalassemia and IDA corresponding normal group. RDW was lower in heterozygote thalassemia in comparing with IDA but this was not significant.

## DISCUSSION

The two main types of hypochromic microcytic anemia are IDA and thalassemia (2,13). They are both common in Iran and differentiation between them for treatment and diagnosis decisions are important (3,4,5). The conventional laboratory tests to diagnosis of IDA are measurement of serum ferritin, iron and TIBC (13). Alkaline and acidic hemoglobin electrophoresis are the most widely used methods for investigating hemoglobin variants.(8) $HbA_2$ value is 3.5-8% in β-thalassemia minor (14). IDA modulates the synthesis of $HbA_2$, resulting in

**Table 1.** Hematologic values and $HbA_2$ levels in normal control group, IDA, α- and β-thalasemia minor and concomitant IDA and β-thalasemia minor in adult individuals, Mashhad, IRAN, 2006.

| Values | Normal | IDA | β-thal. | α-thal* | IDA + β-thal* |
|---|---|---|---|---|---|
| M | 5.47±0.42 | 5.11±1.09 | 6.09±0.66 | 6.1±0.66 | |
| RBC ($x10^6$ ml) | | | | | |
| F | 4.85±0.60 | 4.52±0.68 | 5.45±0.46 | | 5.59±1.87 |
| WBC ($x10^3$ ml) | 6.18±1.25 | 5.83±1.8 | 6.81±1.51 | 6.55±1.33 | 5.11±0.68 |
| M | 15.4±1.5 | 9.1±2.2 | 12.6±1.4 | 12.1±1.8 | |
| Hb (g/dl) | | | | | |
| F | 13.6±0.9 | 9.3±1.9 | 11.2±0.9 | | 11.2±1.1 |
| M | 46.5±3.7 | 33.5±6.9 | 40.9±4.2 | 40.5±4.7 | |
| Hct (%) | | | | | |
| F | 41.3±3.1 | 32.9±5.1 | 36.2±2.9 | | 36.3±1.9 |
| | 83.7±5.1 | 65.7±6.2 | 67.6±4.6 | 67.5±3.6 | |
| MCV(fl) | | | | | |
| | 86.5±5.6 | 72.4±5.9 | 66.6±3 | | 64.8±1 |
| M | 28.2±2.7 | 17.7±2.7 | 20.8±2.1 | 19.9±1.8 | |
| MCH (pg) | | | | | |
| F | 28.6±2.4 | 20.6±3 | 20.5±1.2 | | 20.1±1.9 |
| M | 33.6±1.6 | 26.9±2.4 | 31.1±2.3 | 29.5±1.8 | |
| MCHC (g/dl) | | | | | |
| F | 33±0.9 | 29.1±2.9 | 30.8±1.6 | | 30.9±1.7 |
| RDW (%) | 13.5±1.1 | 18.5±5.4 | 16.5±1.4 | 16.3±1.7 | 17.3±0.9 |
| PLT ($x10^3$ ml) | 242±50 | 282±92 | 239±73 | 238±30 | 238±73 |
| SI (μg/dl) | 95±33 | 32±14 | 97.5±26 | 155±30 | 64±41 |
| TIBC (μg/dl) | 361±65 | 467±92 | 344±62 | 360±46 | 416±38 |
| Ferritin (mg/L) | 51±33 | 7.7±4.2 | 146±158 | 46±38 | 10±5.5 |
| HbA2 (%) | 2.9±0.4 | 2.7±0.6 | 5.6±0.9 | 3.1±1.3 | 4.7±1 |

* Hematologic values shown for α-thalassemia only cover male adults, IDA + β-thalassemia values only cover female adults and $HbA_2$ values, Iron,TIBC, and Ferritin are regardless of age and sex. (M= Male, F= Female, Thal= Thalassemia).

reduce $HbA_2$ levels in patients with IDA. Therefore patients with concomitant IDA and thalassemia can show normal $HbA_2$ levels.

Treatment with Iron in these patients elevates $HbA_2$ to normal levels. Remeasuring $HbA_2$ after IDA treatment is recommended especially when $HbA_2$ levels are borderline ranges (9-11,15). ID may also decrease $HbA_2$ levels in healthy control subjects and even it may reduce the amount of variants hemoglobin in certain hemoglobinopathies (16). Some of other studies have not reported $HbA_2$ reduction as considerable at coincident of these two diseases. (10,17-19). The decrease $HbA_2$ level which composed of a2b2 globin chains in iron deficiency could be due to decrease transcription and/or translation of hemoglobin gene. Another possible explanation is competition between HbA ß chains and $HbA_2$ delta chains in binding the limited quantities of available.

Reduction of $HbA_2$ has been reported to correspond to the severity of anemia. Therefore it is possible that iron deficiency was not sufficiently severe or not sufficiently prolonged to significant reduce the $HbA_2$ level in some patient with β-thalassemia trait. Also mild deficiency of vitamin $B_{12}$/folate may cause an elevation in percentage of $HbA_2$, thus countering the effect of ID (10). Furthermore in some of β-thalassemia mutations and simultaneous inheritance of α and ß thalassemia, $HbA_2$ level may not be increased (6,8). As it is above mentioned we observed a significant decrease of $HbA_2$ levels in IDA patients comparing with normal subjects and also in patients with coincident IDA and β-thalassemia comparing with β-thalassemia minor patients.

These differences especially in later condition was considerable and about 1% (Table 1). Mean hemoglobin value in IDA patients was 9 g/dl. Hemoglobin usually decrease proportionally more than Hct because of hypochromia (20) which was also observed in this study. Although RBC counts were lower in IDA patients in compare to normal subjects but this difference was not significant (Table 1).With considering hemoglobin concentration this condition represents increasing RBC production. MCV, MCH and MCHC decrease in IDA while theirs rate are related to anemia severity and duration (20). Anemia is usually mild to moderate in thalassemia trait. Mean hemoglobin levels are 12.7 g/dl and 10.9 g/dl respectively in male and female. RBC count increase in thalassemia minor and MCV and MCH decrease. MCHC is either normal or decreased (14). These values are similar to the achieved values in this study (table1).

According to a study in kashan - IRAN, most IDA patients have MCV 70 fl and normal RBC count, whereas most patients with thalassemia minor have MCV < 70 fl and increased RBC counts (6). We were not considered significant difference in MCV, between IDA and thalassemia minor.

RDW is valuable in differentiation between IDA and thalassemia minor. It increases at early stages of IDA. RDW is normal or mild increase in thalassemia minor (12,20). As in table 1 of this study, RDW is significantly increased in thalassemia minor comparing with normal subjects but this increase is higher in IDA.

Leukocyte counts are normal or slightly lowered in IDA and platelets may be normal, increased or decreased. We observed normal leukocyte count and increased platelet in IDA patients but the considerable point was increased leukocyte counts in thalassemia minor compare to normal subjects (Table 1).

Conclusion: $HbA_2$ may be decreased in IDA. If an individual with β-thalassemia trait has concomitant severe IDA, the usually elevated $HbA_2$ may be in the normal range. In this instance retesting should be performed after the iron deficiency is corrected.

**REFERENCES**


1. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pediatr Int 44(6):612-6, 2002.
2. Ghaneei M, Movahhedi M, Mirzadeh MJ, Adibi P. Probability for differentiation of blood indices for determination of Iron deficiency in minor Thalassemia based on red cell. Journal of Medical Faculty Guilan University of Medical Sciences, 1997, 22(6): 1-6, 1997. (in Persian with English abstract).
3. hykh Al Eslami H, Asefzadeh S. Causes of hospitalization in Avicina Teaching Center. Journal of Ghazvin University of Medical Sciences, 9: 62-66, 1999. (in Persian with English abstract).

4. Javadzadeh Shahshahani H, Attar M, Taher Yavari M. A study of the prevalence of iron deficiency and its related factors in blood donors of Yazd, Iran. 2003, Transfus Med, 15(4): 287-293, 2005.
5. Ghafouri M, Mostaan Sefat L, Sharifi Sh, Hosseini Gohari L, Attarchi Z. Comparison of cell counter indices in differentiation of Beta Thalassemia minor from Iron deficiency anemia. Blood 2(7): 385-389, 2005. (in Persian with English abstract).
6. Sadr F, Afzali H, Moosavi SGA, Ekinchi H. Prevalence of Iron deficiency anemia and minor beta-Thalassemia and comparison of their red cells indices in volunteers for marriage referred to Golabchi outpatient clinic in Kashan in 1996-1997. Journal of Kashan University of Medical Sciences 12(3): 78-83, 1999. (in Persian with English abstract).
7. Yousefi MH, Arainejad F, Soufizadeh N. Beta Thalassemia trait screening in Sanandaj. Sci J Kur Univ Med Science 1(1): 15-19, 1996. (in Persian with English abstract).
8. Joutovsky A, Hadzi -Nezic J, Nardi MA. HPLC retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: A study of 60000 samples in a clinical diagnostic laboratory. Clin Chem 50:1736-1747, 2004.
9. EL_Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol 24(5): 285-9, 2002.
10. Madan N, Sikka M, Sharma S, Rusia U. Phenotypic expression of hemoglobin A2 in beta-thalassemia trait with iron deficiency. Ann Hematol 77(3):93-6, 1998.
11. Elghetany MT, Katalin B. Erythrocyte disorders. McPherson RA, Pincus MR: Henrys Clinical diagnosis and management by laboratory methods, 21th ed, Saunders company, 2007: 504-507.
12. Perkins SL. Normal blood and bone marrow value in human. Greer JB, Foerster J et al: Wintrobs clinical hematology, 11th ed, Lipincott Williams and Wilkins, 2004: 2697-2702.
13. Chrobak L. Microcytic and hypochromic anemias. Vnitr Lek 47(3):166-74, 2001.
14. Pigbatti CB, Galanello R. The thalassemia and related disorders: Quntitative disorders of hemoglobin synthesis, Greer JB, Foerster J et al: Wintrobs clinical hematology, 11th ed, Lipincott Williams and Wilkins, 2004: 1346-1347.
15. Harthoorn -Lasthuizen EJ, Lindemans J, Langenhuijsen MM. Influence of iron deficiency anaemia on haemoglobin A2 levels: possible consequences for beta thalassaemia screening. Scand J Clin Lab Invest 59(1):65-70, 1999.
16 Steinberg MH. Case report . effects of iron deficiency and the 88 C-T mutation on HbA2 levels in beta-thalassemia. Am J Med Sci 305(5): 312-3, 1993.
17. Sarya AK, Kumar R, Choudhry VP, Tyagi RS, Sehgal AK. Diagnostic efficacy of haemoglobin A2 in heterozygous beta thalassemia. Indian J Med Res 80:203-208, 1984.
18. Hinch life RF, Lilleyman JS. frequency of coincident iron deficiency and beta-thalassemia trait in British Asian children. J Clin Pathol 48:594-595, 1995.
19. Kattamis C, Lagos P, Metaxoton A, Matsanioti SN. Serum iron and unsaturated iron binding capacity in beta-thalassemia trait : their relation to the levels of haemoglobins A, A2 and F. J Med Genet 9:154-159, 1997.
20. Andrews NC. Iron deficiency and related disorders. Greer JB, Foerster J et al: Wintrobs clinical hematology, 11th ed, Lipincott Williams and Wilkins, 2004: 998-999.

**Corresponding Author**
M. R. Keramati
Department of Hematopathology
Emam Reza Hospital
Emam Reza Square
Mashhad
IRAN

Phone: +98 9155199626
E-mail: drkeramati@Gmail.com