

## THE IMPACT OF IRON DEFICIENCY ON HbA2 LEVEL IN BETA THALASSEMIA MINOR IN SULAIMANI NORTHEASTERN IRAQ.

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### ABSTRACT

**Background:** HbA2 plays a key role in screening programs for beta thalassemia because a small increase in this fraction is the most important marker of beta thalassemia heterozygous carriers. The potential impact of coincident iron deficiency on HbA2 based identification of beta thalassemia minor is worrisome issue for screening laboratories, this is especially true for resource-constrained settings where iron deficiency is wide spread and molecular confirmatory tests for borderline HbA2 values may be unavailable.

**Objective:** the aim of this study is to evaluate the effect of iron deficiency on HbA2 level in order to improve the detection of beta thalassemia trait with or without iron deficiency in our population.

**Materials and Method:** In this study 145 individuals were enrolled including normal controls (50), beta thalassemia minor (50) and coincident beta thalassemia with iron deficiency cases (45). Complete blood count, serum iron, total iron binding capacity and HbA2 with HbF estimation were done for every individual.

**Result:** The mean HbA2 level was (2.4± 0.4) in control, (5.2 ± 0.9) in beta thalassemia minor and 5.1± 0.9 in coincident iron deficiency with beta thalassemia minors. All hematological parameters were significantly lower in beta thalassemia minor and coincident iron deficiency with beta thalassemia minor in comparison to the control group. Mean HbA2 level did not show a significant difference in thalassemia minor (5.2±0.9) when compared with the mean HbA2 levels in coincident iron deficiency with beta thalassemia minor.

**Conclusion:** The presence of iron deficiency did not preclude the detection of beta thalassemia minor in our population.

**Key words:** HbA2, thalassemia minor, iron deficiency, anemia.

### INTRODUCTION

The most common hypochromic microcytic anemias are iron deficiency anemia and thalassemia minor (P. Sharma et al, 2015). Thalassemia is the commonest single gene disorder throughout the world and is one of the major public health problems in the endemic regions, such as the Mediterranean countries including Iraq and Kurdistan (Alsamarria A H et al, 2008). The thalassemias are a group of hereditary anemias that result from diminished synthesis of one of the two globin polypeptide chains,  $\alpha$  and  $\beta$ , that combine to form adult hemoglobin A ( $\alpha 2 \beta 2$ ) (Bridges K R et al 2008). They are named according to the globin chain affected or the abnormal hemoglobin produced. Classification of beta thalassemia is based on a description of the molecular mutation or by clinical manifestations. Individuals who are heterozygous for this mutation are beta thalassemia minor, those who are homozygous have beta thalassemia major or a milder thalassemia intermedia (Bain BJ, 2006, Raund D et al, 2005). The beta thalassemia minor (bet thal minor) is usually identified during family studies of patients with more severe forms of thalassemia, population surveys, or more frequently by the chance finding of the characteristic hematologic changes during a routine study (Kaushansky K et al, 2010). The classical phenotype of beta thalassemia minor include an elevated HbA2 (3.4-6 %), relatively high red blood cell count, reduced MCV, MCH levels (Cristina P et al, 2012, Giambona A et al, 2009).

Cases of borderline HbA2 levels, with reduced MCV and MCH, could occur as a result of the inheritance of a mild  $\beta^+$  thalassemia mutation, co-inherited gamma and delta thalassemia minor or due to coexisting pathological conditions such as iron deficiency (Mosca A et al, 2008). Iron deficiency modulates the synthesis of HbA2 because intracellular lack of iron reduces alpha globin chain synthesis which causes posttranslational modification in the assembly of HbA2 tetramer, resulting in reduced HbA2 levels in iron deficiency anemia (Harthoorn-Lasthuizen EJ et al, 1999). Since the diagnosis of beta thalassemia minor depends on raised HbA2 levels, patients with concomitant iron deficiency and beta thalassemia minor may show normal HbA2 level and remain undiagnosed. The identification of beta thalassemia minor is essential for two main reasons, first to differentiate it from iron deficiency since both present as hypochromia and microcytosis, second to prevent beta thalassemia major by early genetic counseling (Hussain Z et al, 2005).

## MATERIALS AND METHODS

A total of 3096 individuals were screened on alternate working days in Sulaimani premarital screen clinic, screening for the concomitant beta thal minor with iron deficiency anemia and beta thal minor among couples attending the clinic. Out of these, 145 individual were enrolled in this study and subsequently divided in three groups; beta thalassemia minor (50), concomitant beta thalassemia minor with iron deficiency (45) and normal healthy individuals as control group (50). Seven ml blood was aspirated from each participant, 3.5 ml into K3 EDTA tube for complete blood count (CBC) by automated analyzer (Beckman Coulter, Fullerton, CA, USA), the analyzer was calibrated with reference methods and had regular quality control program. HbA2 and HbF were estimated by high performance liquid chromatography (VARIANT II, Bio-Rad Laboratories, Hercules, CA, USA), The analyzer was calibrated with reference methods and had regular quality control program. The remaining blood was placed into plain tubes for the assessment of serum iron and total iron binding capacity using BIOLABO kits (manual method). Individuals with low MCV (<80fl) and/or low MCH (< 27pg) with HbA2  $\geq$ 3.5% and transfer in saturation (>16%) were regarded as beta thalassemia minor, while individuals with HbA2  $\geq$ 3.5 % and transferring saturation <16% were diagnosed as having coincident iron deficiency with beta thalassemia minor. On the other hand, subjects showing normal red blood cell indices, normal HbA2 and HbF levels, with normal iron status were regarded as control group. Variables were analyzed by SPSS statistical software (version 21), P value<0.05 was considered significant difference.

## RESULTS

Out of 3096 screened individuals, 120 (3.9%) found to be beta thalassemia minor, the first 50 individuals included for the purpose of the study. Their age ranged from 17-40 years, with a mean of 26.6 $\pm$ 5.3, (29 males and 21 females). At the same time 45 individuals detected to have coincident IDA and beta thalassemia minor, their age ranged from 15-36 years, with a mean of 24.4 $\pm$ 5.1, (8 males and 37 females). The age range in the 50 individuals of the control group was 19-36 years with a mean of 24.6 $\pm$ 3.8, (25 males and 25 females) (table 1)

**Table-1: Age and Sex distribution of cases**

Groups	Age (Mean $\pm$ SD)	Male	Female
B-thal	26.6 $\pm$ 5.3	29	21
IDA+B-thal	24.4 $\pm$ 5.1	8	37
Control	24.6 $\pm$ 3.8	25	25

All hematological parameters were significantly lower in beta thal minor and in beta thal minor with coincident IDA in comparison to the control (Table2).

**Table-2: Comparison IDA and Beta thiol**

Parameters	Mean $\pm$ Std. Deviation		P value	Mean $\pm$ Std. Deviation		P value	Mean $\pm$ Std. Deviation		P value
	B-thal	control		IDA+B-thal	control		B-thal	IDA+ B-thal	
RBC ( $\times 10^6$ /mL)	6.0 $\pm$ 0.5	4.8 $\pm$ 4.0	0.000	5.3 $\pm$ 0.5	4.8 $\pm$ 4.0	0.000	6.0 $\pm$ 0.5	5.3 $\pm$ 0.5	0.000
Hb (g/n/dl)	12.5 $\pm$ 1.2	14.7 $\pm$ 1.2	0.000	11.1 $\pm$ 1.3	14.7 $\pm$ 1.2	0.000	12.5 $\pm$ 1.2	11.1 $\pm$ 1.3	0.000
HCT (%)	39.8 $\pm$ 5.8	43.9 $\pm$ 3.7	0.000	35.3 $\pm$ 4.1	43.9 $\pm$ 3.7	0.000	39.8 $\pm$ 5.8	35.3 $\pm$ 4.1	0.000
MCV (fl)	67.0 $\pm$ 3.3	90.1 $\pm$ 3.8	0.000	66.2 $\pm$ 5.4	90.1 $\pm$ 3.8	0.000	67.0 $\pm$ 3.3	66.2 $\pm$ 5.4	0.338
MCH (Pg)	20.8 $\pm$ 1.2	30.3 $\pm$ 1.5	0.000	20.8 $\pm$ 1.9	30.3 $\pm$ 1.5	0.000	20.8 $\pm$ 1.2	20.8 $\pm$ 1.9	0.970
RDW (%)	15.2 $\pm$ 1.30	12.5 $\pm$ 0.5	0.000	16.7 $\pm$ 4.3	12.5 $\pm$ 0.5	0.000	15.2 $\pm$ 1.30	16.7 $\pm$ 4.3	0.022
HbA2 (%)	5.2 $\pm$ 0.9	2.4 $\pm$ 0.4	0.000	5.1 $\pm$ 0.9	2.4 $\pm$ 0.4	0.000	5.2 $\pm$ 0.9	5.1 $\pm$ 0.9	0.674
S-IRON ( $\mu$ g /dL)	87.7 $\pm$ 24.0	96.8 $\pm$ 17.3	0.033	43.5 $\pm$ 17.0	96.8 $\pm$ 17.3	0.000	87.7 $\pm$ 24.0	43.5 $\pm$ 17.0	0.000
TIBC ( $\mu$ g /dL)	329.7 $\pm$ 63.6	337.7 $\pm$ 49.9	0.486	426.9 $\pm$ 94.0	337.7 $\pm$ 49.9	0.000	329.7 $\pm$ 63.6	426.9 $\pm$ 94.0	0.000
TS (%)	26.9 $\pm$ 6.5	29.0 $\pm$ 5.5	0.081	10.3 $\pm$ 3.7	29.0 $\pm$ 5.5	0.000	26.9 $\pm$ 6.5	10.3 $\pm$ 3.7	0.000

Serum iron transferrin saturation (TS) was lower in beta thal minor ( $26.9\pm 6.5$ ) than control ( $29.0\pm 5.5$ ), but the difference was not significant (P value 0.081), on the other hand, a significantly lower transferrin saturation was discriminating coincident IDA with beta thal ( $10.3\pm 3.7$ ) from control and beta thal minor groups. Likewise, the values of MCV and MCH which were almost the same in the above two mentioned groups. (Table-2). As seen in table (2), the mean HbA2 in beta thal minor was ( $5.2\pm 0.9$ ) and in coincident iron deficiency with beta thal minor ( $5.1\pm 0.9$ ). Both showed significant difference in comparison to the control ( $2.4\pm 0.4$ ), while no significant difference noticed in HbA2 level between the above mentioned groups. In addition RDW was significantly higher in coincident iron deficiency with beta thal minor ( $16.7\pm 4.3$ ) in comparison to beta thal minor cases ( $15.2\pm 1.30$ ).

## DISCUSSION

Accurate and sensitive laboratory diagnosis of beta thal minor is essential for carrier detection and prevention of thalassemia major birth; it also helps in correctly identifying beta thal minor as the cause of microcytic hypochromic anemia and prevents unnecessary iron supplements in these individuals.

Iron status in thalassemia trait is always been an area of interest for study. Earlier it was believed that iron deficiency does not exist in thalassemia trait and some of them suggest a protective effect on iron deficiency in persons with beta thalassemia trait (Hussain Z et al, 2005, Tuphan Kanti Dolai, K.S et al, 2012). The gene frequency of beta thalassemia minor is high and varies considerably from area to area, in Sulaimani province/Iraq previous two studies have shown a prevalence rate of 3.98% (Nasir AS Al-Allawi et al, 2013, Jalal SD, 2010).

In our study no significant difference was found in mean HbA2 levels between beta thal minor and coincident iron deficiency with beta thal minor despite the significant difference in iron status between them. This result was consistent with other studies (Madan N et al, 1998, Srdjan Denic et al, 2013), on the reverse of some other studies that showed a significant difference in HbA2 level between coincident iron deficiency with beta thalassemia minor and beta thalassemia minor (M.Reza Keramati et al, 2015) which could be related to the severity of iron deficiency as a reduction in HbA2 level was noticed in cases with moderate to severe iron deficiency anemia not in mild cases (Majid Yavarian et al, 2011). Reduction of HbA2 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 significantly reduce the HbA2 level in some patients with beta thal. Also mild deficiency of vitamin B12/ Folate may cause an elevation in HbA2, thus countering the effect of iron deficiency (Bilic E et al, 2009). The sample size may be another explanation and the mild deficiency of iron in the included beta thal minor individuals.

RDW increases at early stages of IDA, while it is normal or mildly increased in thalassemia minor (Ntaios G et al, 2007). As in (table-2) RDW was significantly increased in beta thal minor when comparing the values with normal subjects RDW values, but this increase was higher in coincident iron deficiency with beta thal minor which is considered as a reliable red cell indices to discriminate between IDA and haemoglobinopathies, and we could possibly apply it for early detection of IDA in beta thal in future studies.

## CONCLUSION

In this study we found that iron deficiency is likely to have a minuscule, if any, impact on the laboratory diagnosis of beta thalassemia minor by HbA2 level estimation.

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