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The impact of iron deficiency on the diagnostic level of HbA2 in beta- thalassemia trait from the Sulaimani hemoglobinopathies screening program.

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Abstract

Background and objectives: The identification of carriers of beta- thalassemia depends on the detection of a high level of hemoglobin A2. The hemoglobin A2 level is influenced by some elements including iron. The consequence of concomitant iron deficiency on the hemoglobin A2 level is critical in screening laboratories for hemoglobinopathies, particularly in resource-limited ones where molecular identification of hemoglobin A2 levels is unavailable. The aim of this study is to evaluate the consequence of iron deficiency on hemoglobin A2 level to obtain a definite diagnosis of beta- thalassemia trait.

Methods: A total of one hundred forty -five subjects were involved, divided into three groups: (50) healthy controls, (50) beta-thalassemia trait, and (45) coincident iron deficiency with beta-thalassemia trait. Full blood count, Iron status including serum iron with total iron-binding capacity, lastly hemoglobin A2 with hemoglobin F estimation were performed for all enrollees.

Results: The age range in the beta-thalassemia trait group was 7-40 years, with mean of (26.6± 5.3) years, while the concomitant group has an age range of 15-36 years, with a mean of (24.4 ±5.1) years. Meanwhile the age of control group ranged from 19-36 years with a mean of (24.6 ±3.8) years. The hematological parameters were significantly reduced in beta- thalassemia trait and concomitant iron deficiency in comparison to the controls with no significant difference in hemoglobin A2 between beta thalassemia trait and concomitant iron deficiency (5.1± 0.9) and beta-thalassemia minor (5.2 ± 0.9).

Conclusion: No influence of iron deficiency on the identification of beta-thalassemia minor detected in the screened population.

Keywords: Thalassemia trait, Hemoglobin A2, Iron deficiency, Anemia.

Introduction

Microcytic anemia is a globally common detected anemia in medical practice[1]. It may be caused by iron deficiency anemia (IDA), an abnormality in globin genes (hemoglobinopathy or thalassemia), sideroblastic anemia, and anemia of chronic disorders[2]. According to World Health Organization, the primer cause of anemia is a deficiency of iron[3], while thalassemia is the most common single gene disorder which is widely spread throughout the Mediterranean region, Africa, Middle East, Indian

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subcontinent and South East Asia[4]. Thalassemia syndromes occur as a consequence of defect in the amount of globin chain synthesis following recessively inherited mutations in alpha or beta gene clusters. Clinically, beta-thalassemia is classified as minor, major, or intermedia depending on the type of genetic mutation[5]. Detecting an increased level of HbA2 ≥ 3.5% is essential for diagnosing beta-thalassemia trait (BTT) in addition to microcytic, hypochromic red cells, usually associated with mild anemia[6]. For a long time, the existence of iron deficiency with thalassemia syndrome was regarded as an unlikely event. However, studies have shown their existence together[7], meanwhile, HbA2 value had shown lower levels in patients with coexisting BTT + IDA[8]. The incidence of individuals with borderline HbA2 level ranged from 2.2 –16.7% in countries where thalassemia is common[9] that could be due to the type of mutation or to coexisting conditions like iron deficiency[10]. That's why individuals with concomitant IDA and BTT may have normal or borderline HbA2 levels which affect the accuracy of diagnosis. We aimed in this study to evaluate the consequence of IDA on the HbA2 level in the participants.

Material and methods

This is a cross sectional study that was carried out on young couples attending Sulaimani Hemoglobinopathies Screen Clinic / Sulaimani/ Iraq over the period from June 1-December 30th, 2012. A sum of 145 individuals was enrolled and divided into three groups. The first group included 50 cases of BTT, the second group comprised of 45 individuals with concomitant BTT+ IDA, while the third was the control group with 50 normal participants from the abovementioned clinic. A total of 7 ml of blood was aspirated from all participants. The first 3.5 ml was withdrawn into K3 EDTA tube for estimation of complete blood count (CBC) by automated hematology analyzer (Beckman Coulter, Fullerton, CA, USA), and HbA2 and HbF estimation using high-performance liquid chromatography (VARIANT Bio-Rad Laboratories, Hercules, CA, USA). The remaining 3.5 ml of blood was added to the plain tube to estimate total serum iron and total iron-binding capacity by BIOLABO kits manually. This study has included individuals with BTT (low mean corpuscular volume MCV (< 80 fl) and/or low mean corpuscular hemoglobin MCH (<27pg) with HbA2 ≥ 3.5% and transferrin saturation TS (≥16%) and attendees with HbA2 ≥ 3.5% and transferrin saturation < 16% diagnosed as coincident BTT+ IDA[11]. Additionally, the control group comprised of 50 normal individuals from the young couples attending the clinic having normal blood count, normal HbA2 and HbF values and normal iron status were. Cases on iron-replacement therapy was excluded. We used Statistical Package for the Social Sciences (SPSS) software (version 21) for statistical analysis; student's t- test for mean comparison, p-value < 0.05 was regarded significant. The study was approved by the ethical committee at Sulaimani Directorate of Health, and informed consent was obtained from all enrollees.

Results

The studied individuals were divided into three groups. First is BTT (50 individuals), their age ranged between 7-40 years, with a mean of (26.6± 5.3) years, including 29 males and 21 females. The second group was coincident BTT+ IDA (45 individuals) with

an age range of 15-36 years, and a mean of (24.4 ±5.1) years, including 8 males and 37 females. The third; control group (50 individuals), the mean age was (24.6 ±3.8) years with a range of 19-36 years including 25 males and 25 females, Table (1).

Table (1): Demographic data of enrolled individuals

	Age/years (Mean ±SD)	**Male (No.)**	**Female (No.)**
BTT	26.6±5.3	29	21
IDA+ BTT	24.4±5.1	8	37
Control	24.6±3.8	25	25

Individuals who have been diagnosed with BTT had mean RBC count, red cell distribution width (RDW), and HbA2 levels significantly higher in comparison to the control group, while mean hemoglobin (Hb), hematocrit (HCT), MCV, MCH, and serum iron values were significantly lower. In contrast, total iron- binding capacity (TIBC) and the transferrin saturation (TS) did not differ potentially, Table (2).

Table (2): Correlation of laboratory data of BTT and control

Parameters	**Mean ± SD**		**p-value**
	BTT	Control	
RBC ($x10^{12}$/L)	6.0±0.5	4.8±4.0	<0.001
Hb(g/dl)	12.5±1.2	14.7±1.2	<0.001
HCT (%)	39.8±5.8	43.9±3.7	<0.001
MCV(f1)	67.0±3.3	90.1±3.8	<0.001
MCH(pg)	20.8±1.2	30.3±1.5	<0.001
RDW (%)	15.2±1.30	12.5±0.5	<0.001
HbA2 (%)	5.2±0.9	2.4±0.4	<0.001
S-Iron (µg/dl)	87.8±24.0	96.8±17.3	0.03
TIBC (µg/dl)	329.7±63.6	337.7±9.9	0.5
TS (%)	26.9±6.5	29.0±5.5	0.08

Likewise, the mean RBC count, RDW, and HbA2 were also significantly higher in those with coincident BTT+ IDA when compared with corresponding values in the control group. Other RBC parameters and the iron results (serum iron, and TS) were significantly lower, while TIBC was higher, Table (3).

Table (3): correlation of laboratory data of coincident BTT+ IDA and control

Parameters	**Mean ± SD**		**p-value**
	IDA+BTT	Control	
RBC ($x10^{12}$/L)	5.3±0.5	4.8±4.0	<0.001
Hb(g/dl)	11.1±1.3	14.7±1.2	<0.001
HCT (%)	35.3±4.1	43.9±3.7	<0.001
MCV(f1)	66.2±5.4	90.1±3.8	<0.001
MCH(pg)	20.8±1.9	30.3±1.5	<0.001
RDW (%)	16.7±4.3	12.5±0.5	<0.001

HbA2 (%)	5.1±0.9	2.4±0.4	<0.001
S-Iron (µg/dl)	43.5±17.0	96.8±17.3	<0.001
TIBC (µg/dl)	426.9±94.0	337.7±49.9	<0.001
TS (%)	10.3 ±3.7	29.0±5.5	<0.001

Finally, considering RBC indices in BTT group and coincident BTT+ IDA group, it's clear that mean RBC count, Hb, HCT were significantly higher in BTT group than in those with coincident BTT+ IDA group, Table (4), unlike MCV, MCH, and HbA2 which showed no significant difference. Among the RBC indices in BTT only the RDW was lower (15.25±1.30 vs 18.7±4.3) respectively. In contrast, serum iron and TS in BTT was almost twice that in coincident BTT+ IDA, while TIBC was lower (p-value 0.0001).

Table (4): correlation of laboratory data BTT+ IDA and BTT

Parameters	**Mean ± Std. deviation**		**p-value**
	IDA+ BTT	BTT	
RBC ($x10^{12}$/L)	5.3±0.5	6.0±0.5	<0.001
Hb(g/dl)	11.1±1.3	12.5±1.2	<0.001
HCT (%)	35.3±4.1	39.8±5.8	<0.001
MCV(f1)	66.2±5.4	67.0±3.3	0.33
MCH(pg)	20.8±1.9	20.8±1.2	0.97
RDW (%)	16.7±4.3	15.2±1.30	0.02
HbA2 (%)	5.1±0.9	5.2±0.9	0.67
S-Iron (µg/dl)	43.5±17.0	87.8±24.0	<0.001
TIBC (µg/dl)	426.9±94.0	329.7±63.6	<0.001
TS (%)	10.3 ±3.7	26.9±6.5	<0.001

Discussion

A high frequency of beta thalassemia carriers has been reported in Iraq ranging from 3.7-4.5% in different areas of the country[12]. Sulaimani is one of provinces where a high BTT frequency of 4.1% has been reported[13]. Since BT major has been a significant problem for both health authorities and affected families in Sulaimani, great efforts had been made for continuous and accurate detection of carrier states through establishing a mandatory premarital screening program from 2008[14]. Moreover, IDA and thalassemia syndrome are the most common causes of hypochromic microcytic anemia, besides preventing thalassemia major, a proper differentiation of IDA from BTT would avoid irrelevant use of iron-supplements in cases of microcytic anemia, and eventually prevents further aggravation of anemia in BTT individuals having iron deficiency. Accurate and sensitive estimation of HbA2 is vital for BTT detection. Additionally, borderline or low HbA2 level has been attributed to coexisting IDA among other causes[15] that would render the recognition of BTT difficult among screening population[9, 16]. In our study, HbA2 value didn't vary significantly between coincident IDA+ BTT and BTT in accordance with other studies[8, 17-18], although the difference in iron status was significant, (Table 4). This result apposed some previous studies where HbA2

level was significantly lower in coincident IDA+ BTT in comparison to BTT[19- 21]. Variation fromprevious reports might be explained by variation in the degree of iron deficiency and hence the severity of anemia, as a reduction in HbA2 level was reported in anemias of moderate to severe degree rather than mild anemia[22]. Some factors may emend the effect of iron deficiency on the level of HbA2 like the deficiency of B12 and folate[23]. Another important factor which should be considered in this variable result is the variation in sample size. Moreover, a significant difference was noticed in RDW values between the BTT and coincident IDA+BTT in accordance with other studies that revealed an increase of RDW in early stages of iron deficiency with no or mild raised levels in beta-thalassemia minor[24]. This might make this index a reliable and simple indicator to differentiate between iron deficiency and thalassemia in recourse-limited laboratories[25].This study has the limitation of mismatched gender between control and coincident IDA/BTT group that might have impacted the comparisons between the above groups, in particular when it comes to Hb/RBC/Hct as they differ in males and females. However, the scope of this study was to estimate the effect of iron deficiency on HbA2 diagnostic value and not the comparison of CBC parameters between different enrolled categories.

Conclusion

Iron deficiency did not impede the recognition of classical BTT in our screened population. In addition, RDW is a suitable index for discrimination between IDA and BTT.

Conflicts of interest

There were no conflicts of interest.

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