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Diagnosis of patients with hemoglobinopathies including α-thalassemia in a laboratory with limited resources

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Abstract:

BACKGROUND: Diagnosis of α-thalassemia can be challenging as it is clinically insignificant in the majority of patients who are presented with one or two α-gene deletion, it cannot be always suspected from the red cell indices, and the confirmatory tests of α-thalassemia are not readily available, applied, and/or provide conclusive results. The importance of diagnosis of α-thalassemia is mainly to identify the patients with α^0-thalassemia, hemoglobin (Hb) H disease, Hb Bart's hydrops fetalis, and to identify the ameliorating effect that the coinheritance of α-thalassemia may have on the clinical phenotype of patients with HbS and other structural β-globin chain variants and/or patients with beta-thalassemia. The aim of this study is to evaluate the presentation and diagnosis of patients with α-thalassemia in a cohort of Iraqi patients with hemoglobinopathies.

PATIENTS, MATERIALS, AND METHODS: This is a prospective cohort study that included 216 patients diagnosed with different types of hemoglobinopathies from June 2016 to October 2019. For each patient after reviewing the cause of presentation and family history of thalassemia, blood samples were sent for complete blood count, including calculated absolute reticulocyte count, peripheral blood smear, Hb H preparation, serum iron and total iron-binding capacity, sickling test, and if indicated Hb high-performance liquid chromatography (HPLC) (using BioRad D10). Family study was requested for several patients.

RESULTS: Male:female for patients with α-thalassemia is 1:1.25. Consanguinity and family history of α-thalassemia are present in 85.2% and 51.9%, respectively. Out of 216 patients with different types of hemoglobinopathies, 25% are diagnosed with isolated or copresence of α-thalassemia. Low/lower normal HbA_2% is the most sensitive parameter for the diagnosis of α-thalassemia.

CONCLUSIONS: The most sensitive parameter for the diagnosis of α-thalassemia in this study is the low/lower normal HbA_2% in patient with normal iron study. Other parameters such as the presence of golf-ball red cells in Hb H preparation and/or presence of HbH wave in Hb HPLC can be helpful but are only seen in smaller fraction of patients.

Keywords:

α-thalassemia, hemoglobinopathy, limited-resources

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Introduction

Thalassemia is an inherited disease resulting from the reduced rate of synthesis of one or more of the globin chains, and hence, a reduced rate of synthesis of the hemoglobin (Hb), with an excess of the normal globin chain contributing to the pathological effect, causing either damage to erythroid precursors and ineffective erythropoiesis or damage to mature erythrocytes and hemolytic anemia.

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Table 1: Demographic presentation of patients with thalassemia and hemoglobinopathies

Parameters	Patients with α-thalassemia (*n*=54), *n* (%)	All other patients (*n*=162), *n* (%)
Gender		
Male	24 (44.4)	64 (39.5)
Female	30 (55.6)	98 (60.5)
Age at presentation (years)		
<1-10	11 (22.2)	86 (53.1)
10-20	19 (33.3)	25 (29.6)
20-30	14 (25.9)	17 (11.1)
>30	10 (18.6)	10 (6.2)
Consanguinity		
Yes	46 (85.2)	134 (82.7)
No	8 (14.8)	28 (17.3)
Family history		
Yes	29 (51.9)	142 (87.7)
No	25 (48.1)	20 (12.3)

Table 2: Classification of patients with thalassemia and/or hemoglobinopathies

Type (*n*=216)	*n* (%)
Possibility of one or two α-gene deletion thalassemia	18 (8.3)
HbH disease	3 (1.4)
HbAS with possible one/two gene deletion α-thalassemia	31 (14)
Combined HbAS and HbH disease	2 (0.9)
β-thalassemia minor	112 (52)
β-thalassemia major	3 (1.4)
HbSS	7 (3.2)
HbS $\beta^{(0)}$-thalassemia	4 (1.9)
HbAS	10 (4.6)
HbS $\beta^{(+)}$-thalassemia	14 (6.5)
HbAC	2 (0.9)
HbAE	3 (1.4)
HbEE	1 (0.5)
HbAD	1 (0.5)
δβ thalassemia trait	4 (1.9)
δβ thalassemia disease	1 (0.5)

HbH=Hemoglobin H, HBAS=Sickle cell trait

The α-thalassemia results from the reduced rate of synthesis of α-globin chains. The severity of α-thalassemia is very variable. At one end it is an asymptomatic condition, resulting from the deletion of one α-gene, which may produce a trivial abnormality in the blood count. At the other end is Hb Bart's hydrops fetalis, which is incompatible with life, resulting from the deletion of all four α-genes and a consequent total lack of Hb synthesis.

When both α-genes on a single chromosome are deleted, the designation α^0-thalassaemia is used. When only one of the two α-genes on a chromosome is deleted, then the designation α^+-thalassemia is used. Fortunately, Iraq is a country where α^0-thalassaemia is rare.[1]

In Iraq, the presumed prevalence of α-thalassemia is 1%,[2] and the diagnosis usually happens as an incidental finding, typically, the patient will present with thalassemic red cell indices, including increased/upper normal red cell count, very low mean cell volume (MCV), and mean cell hemoglobin (MCH) for the level of Hb, normal/near normal red cell distribution width with/without mild/moderate anemia and normal iron study. Hb H preparation may show the presence of golf-ball cells in some patients, especially those with HbH disease and some patients with 2 α-genes deletion; however, the absence of these inclusions should not hinder the presumptive diagnosis of α-thalassemia. Hb high-performance liquid chromatography (Hb HPLC) may show HbH wave, especially in young kids and this will appear as an early double wave, but this wave may not always be apparent in adults, leaving only low/lower normal HbA_2% as a clue to the diagnosis from the Hb HPLC result. Moreover, Hb HPLC findings may be within the normal pattern, because reduced α-globin chain production can result in relatively equal reduction of HbA, HbA_2, and HbF percentages. A family history and study may also be helpful.[3]

Provisional diagnosis of α(+)-thalassemia is generally accepted in certain ethnic groups, including Iraqi/Arab, if the clinical presentation and laboratory findings are consistent with the presence of thalassemia, and exclusion of possibility of β-thalassemia and iron-deficiency anemia using Hb HPLC and serum ferritin and/or serum iron and total iron-binding capacity (TIBC), respectively.[1]

Confirmation of the diagnosis of α-thalassemia is by DNA analysis; however, this test is available for only a small group of highly selected patients in Iraq, usually within the limit of a research project, due to financial restrictions.

The aim of this study is to evaluate the presentation and diagnosis of patients with α-thalassemia in a cohort of Iraqi patients with hemoglobinopathies.

Patients and Methods

This is a prospective cohort study that included 216 patients diagnosed with different types of hemoglobinopathies from June 2016 to October 2019; the patients are presented to a private laboratory for an *ad-hoc* testing, premarriage screening, or referred from different laboratories for the second opinion. Family study was requested for several patients, and if appropriate, the pertinent results of these relatives were included in the study as a separate patient.

Written informed consent was collected from one or both parents according to the declaration of Helsinki, and the study is approved by the designated scientific committee in Al-Mamoon University College which is responsible for approving the ethical and scientific aspects of research projects.

For each patient after reviewing the cause of presentation/referral and family history of hereditary anemia/thalassemia, blood sample was sent for automated complete blood count (CBC), including calculated absolute reticulocyte count (calculated from multiplying manually counted reticulocyte percentage by the red blood cell count from the patient's CBC), peripheral blood smear, HbH preparation, serum iron and TIBC, sickling test, and if indicated Hb HPLC (using BioRad D10-hemoglobin Testing System, USA).

Screening for G6PD-deficiency, using methemoglobin reduction test, was used for male patients only, and patients with deficient enzyme were excluded from the results of this study. Six patients with possible diagnosis of α-thalassemia were excluded from this study, because they had copresence of severe iron-deficiency anemia and did not show up for a follow-up Hb HPLC after a supposed 3 months course of oral iron treatment.

Results

Male:female ratio for patients with α-thalassemia is 1:1.25, and for other patients with β-thalassemia and/or hemoglobinopathies is 1:1.53.

Table 3: Hemoglobin H preparation and hemoglobin high-performance liquid chromatography findings in patients with α-thalassemia (*n*=54)

Parameter	Result	*n* (%)
HbH preparation finding golf-ball cells	Yes	13 (24)
	No	41 (76)
Presence of HbH wave by Hb HPLC	Yes	10 (18.5)
	No	44 (81.5)
HbA_2 ≤1.9% by Hb HPLC	Yes	19 (90.5)
	No	2 (9.5)
HbA_2 ≤2.1% by Hb HPLC	Yes	21 (100)
	No	0 (0)
HbF >5% by Hb HPLC*	Yes	0 (0)
	No	21 (100)

*Only patients with isolated α-thalassemia are included. The choice of 5% cutoff value is related to the use of BioRad D10 platform. HPLC=High-performance liquid chromatography, Hb=Hemoglobin, HbH=Hemoglobin H

Demographic data of patients with thalassemia and hemoglobinopathies are presented in Table 1, classification of patients with thalassemia and/or hemoglobinopathies is presented in Table 2, hemoglobin H preparation and Hb HPLC findings in patients with α-thalassemia are presented in Table 3, laboratory results of patients with thalassemia and/or hemoglobinopathies, with copresence of α-thalassemia are presented in Table 4, and laboratory results of patients with thalassemia and/or hemoglobinopathies, and no evidence of α-thalassemia are presented in Table 5.

Discussion

Diagnosis of hemoglobinopathy in Iraq is almost always presumptive, and it is based on the results of CBC, including reticulocyte count/percentage, blood-film morphology, some ancillary tests such as Hb H preparation, objectively proving the presence of HbS using sickling test and identifying some physical characteristics of hemoglobin using the HPLC technique. These tests usually suffice to diagnose thalassemia and sickle-cell trait (HbAS) and anemia (HbSS).

Presumptive identification of structural hemoglobinopathies, other than HbAS or HbSS, was sickling test and it is the second technique, should be based on a minimum of two techniques that are of different principles in the diagnosis of hemoglobinopathy; however, there can be inadequate funding to perform more than one technique or to extend testing to an appropriate family study.[3]

Definitive diagnosis usually requires DNA analysis using Gap-PCR, Sanger sequencing α-genes with/without multiplex ligation-dependent probe amplification (MLPA), and Sanger-sequencing β-genes with/without MLPA for α- and β-thalassemia, respectively. Family genetic studies are also of considerable importance in elucidating the nature of disorders of Hb in some difficult cases. More advanced techniques such as array comparative genome

Table 4: Laboratory results of patients with thalassemia and/or hemoglobinopathies, with copresence of α-thalassemia (*n*=54)

Parameters (unit)	Mean±SD			
	α-thalassemia (*n*=18)	HbH (*n*=3)	HAS + α-thalassemia (*n*=31)	HbAS + HbH (*n*=2)
Hb (g/dl)	12.2±2.5	9.8±3.3	10.5±3.3	7.3±2.9
PCV (%)	37±7.4	30.2±11	34.1±9.5	21.4±8.8
RBC ($\times10^{12}$/l)	5.6±1.1	5.5±0.9	5.2±0.8	4.4±1.4
Reticulocytes count	89±37	166±43	143±59	183±32
MCV (fl)	65±6.6	51±6.2	67±4.7	78±11
MCH (pg)	18.7±4.5	17.8±2.8	21.2±3.3	23.3±3.2
WBC ($\times10^{9}$/l)	5.2±2.2	7.7±5.1	6.0±3.6	8.6±4.5
Platelet ($\times10^{9}$/l)	311±96	447±202	267±111	232±121

Hb=Hemoglobin, PCV=Packed-cell volume, WBC=White blood cell, RBC=Red blood cell, MCV=Mean cell volume, MCH=Mean cell hemoglobin, SD=Standard deviation, HbH=Hemoglobin H

Table 5: Laboratory results of patients with thalassemia and/or hemoglobinopathies, with no evidence of α-thalassemia

Parameters (unit)	Mean±SD										
	β-thalassemia minor	β-thalassemia major	HbSS	HbSβ^0	HbAS	HbSβ$^+$	HbAC	HbAE	HbEE	HbAD	δβ-thalassemia trait	Homozygous δβ-thalassemia
Hb (g/dl)	11.1±2.3	5.2±1.1	8.3±2.1	6.8±1.5	10.8±3.7	8.1±2.1	11.6±1.3	10.9±3.1	10.7	11.8	12.2±0.9	11.6
PCV (%)	33.8±6.1	16.1±3.0	25.1±6	21±4.2	32.6±11.9	24.6±6.6	35.4±4	33.1±9.2	35	36	36.4±3.2	34.2
RBC (×10^{12}/l)	5.7±1.2	2.6±1.2	3.2±1.4	4.98±1.1	3.9±1.6	4.05±1.4	4.8±1.3	4.36±1.4	5.8	4.9	4.88±1.4	5.0
Reticulocytes count	91.2±68.4	39±20.8	54±23	67±15	41±28	84±24	62±25	56±33.7	87	43	32±12	66
MCV (fl)	62±9.3	69.3±7.2	73±3.7	60±3.5	76±3.9	63.2±7.4	77.4±2.6	80.3±6.3	63	80.2	70.9±2.2	68.4
MCH (pg)	19.9±4.7	20.1±3.6	26.1±2.5	19.1±1.2	23.8±1.9	19.3±3.5	27.3±1.8	25.5±3.1	19	26.9	24.1±3.0	23.8
WBC (×10^9/l)	7.2±3.4	8.8±5.2	7.1±4.3	8.9±2.2	9.6±2.7	7.7±4.4	4.9±0.6	6.4±2.1	6.6	7.3	5.54±1.3	15.9
Platelet (×10^9/l)	423±166	391±117	323±56	385±23	366±83	375±89	196±30	315±77	288	238	206±47	200

Hb=Hemoglobin, PCV=Packed-cell volume, WBC=White blood cell, RBC=Red blood cell, MCV=Mean cell volume, MCH=Mean cell hemoglobin, SD=Standard deviation, HbH=Hemoglobin H

hybridization, target locus amplification, and next generation sequencing can be helpful in practice in the near future.[4]

The diagnosis of α-thalassemia can be challenging because; 1st: it is usually clinically insignificant in the majority of patients who are presented with one or two α-gene deletion; 2nd: it cannot be always suspected from the red cell indices; and 3rd: the confirmatory tests of α-thalassemia are not readily available, applied, and/or provide conclusive results.[3,5]

The importance of the diagnosis of α-thalassemia is mainly to identify the patients with α^o-thalassemia, HbH disease, Hb Bart's hydrops fetalis, and to identify the good-modifying effect that the coinheritance of α-thalassemia may have on the clinical phenotype of patients with HbS, and other structural β-globin chain variants, and/or patients with beta-thalassemia.[1]

The difficulty to firmly diagnose α-thalassemia basically arise because of concurrent reduction of all main Hb waves in Hb HPLC technique, including HbA, HbA_2, and HbF, as each molecule of these Hb contain two α-globin chains. However, greater predilection of α-globin chains to β^A- and γ-globin chains than to δ-chains can result in low/below normal HbA_2 percentage.[3]

The presence of α-thalassemia in Iraq can be considered relatively useful, as it is usually present with one or two α-gene deletion, i.e., clinically insignificant *per se*, and the α^o-thalassemia, and hence, hydrops fetalis are very rare.[1] Moreover, it is relatively common to diagnose copresence of α-thalassemia in patients with sickle-cell trait with the presence of α-gene deletion is considered as an ameliorating factor. The diagnosis of copresence of α-thalassemia in patients with sickle-cell anemia or disease, and β-thalassemia can be difficult, though few patients may be suggested to have α-thalassemia through the family study.[3,5]

Suggesting the presence of isolated α-thalassemia with 1- or 2-α gene deletion is one of exclusion of both iron-deficiency anemia and β-thalassemia minor in a patient with thalassemic red cell indices, including increased/upper normal red cell count and low MCV and MCH for the level of Hb, with/without anemia. While the diagnosis of HbH and combined α-thalassemia and β-globin chain structural hemoglobinopathy is based on more objective findings, including the presence of golf-ball red cells in HbH preparation, and Hb HPLC findings of early double-wave consistent with HbH and reduced HbA_2% for HbH disease, and reduced percentage of heterozygous β-globin chain variant below the expected 35% for β-globin chain structural hemoglobinopathies.[4] The diagnosis of

α-thalassemia in the presence of homozygous or even compound heterozygous β-globin chain structural hemoglobinopathy can prove difficult in practice and is usually not counted for though it may be clinically an ameliorating factor.[3,5]

In this study, the age at presentation appears to be older for patients with α(+)-thalassemia and with lower rate of recorded family history of same condition, in comparison with patients with HbH disease and β-thalassemias, and this may be explained by that α-thalassemia is more difficult to diagnose and less clinically significant.

Out of 216 patients with different types of hemoglobinopathies included in the current study, 25% are diagnosed with isolated or copresence of α-thalassemia. Hence, it appears that the prevalence of α-thalassemia is more than what thought before as only 1%.[2]

The most sensitive parameter for the diagnosis of α-thalassemia in this study is the low/lower normal HbA_2% in a patient with normal iron study, 90.5% of patients with HbA_2% ≤1.9, and 100% with HbA_2% ≤2.1%. Other parameters such as the presence of golf-ball red cells in HbH preparation and/or presence of HbH wave in Hb HPLC can be helpful but are only seen in smaller fraction of patients.

Patients with possible -$\alpha^{3.7}$ deletion which is proved to be present in significant proportion in Saudi Arabia neighboring Iraq, and such patients are left with only clinical label of possible thalassemia as they require genetic study to prove its presence. However, the presence of microcytosis and/or thalassemic red cell indices in a patient with normal iron-study and Hb HPLC result can point to the diagnosis of -$\alpha^{3.7}$ deletion, especially when homozygous -$\alpha^{3.7}$ deletion is present.[6]

The use of BioRad D10 Hb HPLC has the main advantage of being much cheaper than using more standard *in vitro* diagnostic Hb HPLC machine like BioRad Variant II; however, the disadvantage of not being able to clearly differentiate between the early Hb fraction waves that appear before HbA_0 may discourage using it by the less experienced hematopathologists.[1]

Conclusions

The most sensitive parameter for the diagnosis of α-thalassemia in this study is the low/lower normal HbA_2% in patient with normal iron study. Other parameters such as the presence of golf-ball red cells in HbH preparation and/or presence of HbH wave in Hb HPLC can be helpful but are only seen in smaller fraction of patients.

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Conflicts of interest
There are no conflicts of interest.

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