Screening for β-thalassaemia carriers in Egypt: significance of the osmotic fragility test

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تحرِّي حَمَلة الثلاسيمية بيتا في مصر: أهمية اختبار الهشاشة التناضُحية

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الخلاصة: لتقدير معدل حَمَلة الثلاسيمية بيتا في مصر، وللتعرُّف على مدى دقة اختبار التحرِّي الجموعي، أجرت الباحثات اختبارات على ألف طفل تتراوح أعمارهم بين 5 و16 عاماً، مِمَّن تم اختيارهم عشوائياً من مختلف المناطق الجغرافية في مصر. وقد لُوحظ صغر الكريات الحمراء لدى 412 من المشاركين. كما أن اختبار الهشاشة التناضُحية كان إيجابياً في 81.1٪ من حَمَلة الثلاسيمية بيتا البالغ عددهم 90 طفلاً. أما في المجموعة غير المحدَّدة (وعددهم 12 طفلاً) فإن الاختبار كان إيجابياً لدى 83.3٪، وفي 310 من المساهمين الذين يعانون من عَوَز الحديد كان الاختبار إيجابياً لدى 63.9٪. وكان معدل حَمَلة الثلاسيمية بيتا يساوي أو يزيد على 9٪. وقد تبيَّن أن حديد المصل، وصِغَر الكريات الحمراء، ومستوى الهيموغلوبين A2، والإشباع بالترانسفيرين، تمثِّل اختبارات دقيقة لكشف الحَمَلة. أما اختبار الهشاشة التناضُحية بأنبوب واحد فقد كانت حساسيته 87٪ ونوعيَّته 34٪، مِمَّا يدلُّ على أن لهذا الاختبار فائدة محدودة في برنامج التحرِّي الجموعي في مصر، حيث يشيع فقر الدم بعَوَز الحديد.

ABSTRACT To estimate β-thalassaemia carrier rate and to determine an accurate mass screening test, we tested 1000 randomly selected children aged 5–16 years from different geographical areas of Egypt. Microcytosis was present in 412 participants. The osmotic fragility test was positive in 81.1% of the 90 β-thalassaemia carriers; in the indeterminate group (12 participants), the test was positive in 83.3%; in the 310 who were iron deficient, the test was positive in 63.9%. β-thalassaemia carrier rate was ≥ 9%. Serum iron, microcytosis, HbA2 level and transferrin saturation were accurate tests for detecting carriers. For the one-tube osmotic fragility test, sensitivity was 87.0% and specificity 34.1%; the test has limited use for a mass screening programme in Egypt, where iron deficiency is prevalent.

Dépistage des porteurs de la β-thalassémie en Égypte : signification du test de fragilité osmotique

RÉSUMÉ Afin d'estimer le taux de portage de la β-thalassémie et d'identifier un test de dépistage de masse fiable, nous avons sélectionné au hasard 1000 enfants âgés de 5 à 16 ans originaires de différentes régions d'Égypte. Une microcytose était présente chez 412 participants. Le test de fragilité osmotique était positif chez 81,1 % des 90 porteurs de la β-thalassémie. Dans le groupe « indéterminé » (12 participants), ce test a été positif dans 83,3 % des cas, tandis qu'il l'a été dans 63,9 % des 310 cas de carence martiale. Le pourcentage de porteurs de la β-thalassémie était ≥ 9 %. La sidérémie, la microcytose, l'hémoglobinémie A2 (HbA2) et la saturation de la transferrine se sont avérés être des tests fiables pour le dépistage des porteurs de cette anomalie. La sensibilité et la spécificité du test de fragilité osmotique monotube ont été respectivement de 87,0 % et 34,1 %. L'utilisation de ce test dans le cadre d'un programme de dépistage de masse en Égypte est limitée compte tenu de la prévalence de la carence martiale dans ce pays.

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Introduction

Thalassaemia syndromes are the most common single-gene disorder worldwide: about 3% of the world population (150 million) carries the β-thalassaemia genes [*1*].

In Egypt, β-thalassaemia is the most common genetically-determined, chronic, haemolytic anaemia. The actual number of patients surviving to date is not, however, available [*2*].

The economic and social cost of the disease is high owing to the patient's lifelong need for monthly blood transfusions and treatment with iron chelating agent. If there is no concomitant reduction in the number of new thalassaemia major births, there will be a cumulative increase in numbers requiring treatment [*3*]. Screening programmes for detection of β-thalassaemia trait, together with prenatal diagnosis and elective abortion of homozygous fetuses, allow couples at risk to avoid having a homozygous thalassemic child [*4*].

Screening for β-thalassaemia is difficult, mainly because of heterogeneity of β-thalassaemia and the absence of a single pathognomonic finding to cover all variants. Despite these difficulties, many attempts have been made to establish screening tests and to aid in the differentiation of various forms of microcytic anaemia, especially the most common, iron deficiency anaemia [*1*]. In some areas the birth of homozygotic infants has fallen dramatically [*5*].

The most reliable methods for diagnosis of thalassaemia trait include quantitative determination of haemoglobin A2 (HbA2), haemoglobin F (HbF), globin chain synthetic ratios and DNA studies for specific mutations. These methods are accurate but too expensive for initial mass screening [*6*]. Since thalassaemia is almost invariably associated with microcytosis and significant hypochromia, determination of red cell index has been used as a preliminary indication of thalassaemia trait [*7*].

In Egypt, no definite national screening programme has yet been developed for detection of β-thalassaemia carriers [*8*].

The aim of our study was to determine the carrier rate of β-thalassaemia in Egypt, and to determine the most economic and accurate test for a mass screening programme.

Methods

This study was carried out during the period September 2004–April 2005. The participants comprised 1000 school-age children from different geographical areas, 40% from Upper Egypt and 60% from Lower Egypt. The children were randomly selected from healthy siblings of patients at the new Cairo University Children's Hospital as well as children who were attending the surgical department of the hospital for minor procedures. This hospital is the largest referral hospital in the country; patients are referred from all areas of Egypt. The participants had no signs or symptoms suggesting haematological disease and no family history of any haematological disease.

Mean age was 10 (standard deviation 3) years. Informed consent was obtained from the children's guardians for all participants. There were no refusals to participate.

One hundred β-thalassaemia carriers, who were parents of known β-thalassaemia patients, were enrolled in Group 4 as controls. They were randomly selected while visiting the haematology clinic of the new Cairo University Children's Hospital for follow-up appointments with their children (thalassaemia patients). This hospital is the biggest referral centre for haematological diseases in Egypt. There were no refusals to participate.

Blood samples (5 mL) were taken from all participants and tested at the clinical pathology laboratory in the new Cairo University Children's Hospital. A 1 mL aliquot of venous blood was mixed with 1.2 mg EDTA to do a complete blood count for all participants using an electronic Coulter counter (Sysmex KX-21N) and to assess haemoglobin, haematocrit, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH).

For participants whose results indicated microcytosis, i.e. MCV < 80 fL [*9*] and/or hypochromia, i.e. MCH < 27 pg [*9*], a second 6 mL venous blood sample was taken. The sample was split into 2 test tubes and the following tests were carried out immediately.

- Tests for iron status
  - serum iron level by automated analyser (Beckman Coulter Synchron CX9 PRO); normal range 70–200 µg/dL
  - total iron-binding capacity (TIBC) by automated analyser; normal range 250–435 µg/dL
  - transferrin saturation (TS = serum iron/TIBC × 100); normal range 20%–45%.
- Tests for β-thalassaemia carrier detection
  - HbA2%, the gold standard test used in this study, by microcolumn chromatography (Helena Beta-Thal HbA2 Quik Column, cut-off 3.5%) [*10*].
  - haemoglobin F (HbF%) by cellulose acetate electrophoresis at pH 8.4 (cut-off 1.0%) [*10*]
  - one-tube red cell osmotic fragility test with 0.36% buffered saline solution [*11*].

To perform the osmotic fragility tests 0.3 mL of whole blood was added to 9 mL 0.36% buffered saline. Each tube was mixed well by inverting 5 times. After 10 minutes, the tube was inspected visually in a proprietary test tube holder with a striped background. If the stripes were clearly visible, indicating complete lysis, the test was read as negative. If turbidity caused the lines to be blurred, the test was considered positive. Equivocal results were those in which there was a very fine cloudiness in the tube and the edges of the lines were slightly blurred. All equivocal or definite positive results were regarded as positive, indicating the need for further investigation.

The numerical data were presented as mean and standard deviation. The Student *t*-test (unpaired-*t*) was used to compare between groups of numerical data. *P*-value < 0.05 was considered statistically significant. Diagnostic properties such as sensitivity, specificity and predictive value were used for data analysis [*12*].

Results

The complete blood count testing of the 1000 children we screened revealed that 412 (41.2%) showed microcytosis (MCV < 80 fL). These participants were divided into groups according to their HbA2 level, HbF level and iron status.

- Group 1, the β-thalassaemia carrier group, had high levels of HbA2 (> 3.6%) and normal levels of the iron parameters studied. Three (3.3%) had high HbF levels (Table 1). This group comprised 90 children (9%).
- Group 2, the indeterminate group, comprised 12 children (1.2%) with borderline levels of HbA2 (range 3.3%–3.5%), low transferrin saturation and low serum iron, but with normal TIBC (Table 1).
- Group 3, the iron deficiency group, comprised 310 children (31.0%) with normal levels of HbA2 (range 1.3%–2.4%), low

Table 1 **Comparison of blood test results for 3 groups of children with microcytosis and a control group**

Test	Children with microcytosis (*n* = 412)			Controls (*n* = 100)
	Group 1 (*n* = 90)	Group 2 (*n* = 12)	Group 3 (*n* = 310)	Group 4
Hb (g/dL)				
Range	8.7–12.9	8.4–10.8	7.0–11.0	10.1–13.8
Mean (SD)	11.0 (0.9)	9.9 (0.8)	9.1 (1.1)	11.6 (0.9)
RBCs (× 10^{12}/L)				
Range	4.1–5.3	3.8–4.2	3.1–4.9	4.5–6.6
Mean (SD)	4.8 (0.2)	4.0 (0.1)	3.9 (0.2)	5.4 (0.4)
Hct (%)				
Range	28.2–37.5	26.0–30.2	21.3–33.2	30.4–43.6
Mean (SD)	32.7 (2.2)	28.7 (1.3)	28.0 (2.3)	36.3 (3.1)
MCV (fL)				
Range	64.0–72.0	68.0–72.0	66.0–74.0	53.0–72.0
Mean (SD)	67.7 (2.1)	69.6 (1.2)	71.0 (2.1)	67.3 (3.1)
MCH (pg)				
Range	19.30–25.0	22.0–26.0	19.0–31.10	17.30–27.8
Mean (SD)	22.9 (1.1)	24.2 (1.3)	23.3 (1.5)	21.6 (1.5)
MCHC (g/dL)				
Range	30.0–35. 0	32.2–35.0	28.0–35.4	30.0–34.4
Mean (SD)	33.6 (1.1)	34.0 (1.0)	32.2 (1.4)	32.1 (1.3)
Serum iron (μg/dL)				
Range	69.0–135.0	27.0–47.0	20.0–54.0	88.0–167.0
Mean (SD)	106.4 (13.0)	38.5 (6.0)	36.2 (5.9)	115.0 (15.3)
TIBC (μg/dL)				
Range	232.0–384.0	314.0–379.0	223.0–462.0	253.0–429.0
Mean (SD)	322.8 (33.1)	337.1 (19.2)	340.6 (32.7)	334.5 (29.4)
TS (%)				
Range	22.7–40.0	8.0–15.0	5.40–16.0	28.0–47.0
Mean (SD)	33.0 (2.8)	11.7 (1.9)	10.7 (1.6)	34.3 (3.2)
HbA2 (%) (column chromatography)				
Range	3.9–6.0	3.3–3.5	1.3–2.4	3.8–6.2
Mean (SD)	4.8 (0.5)	3.4 (< 0.1)	1.9 (0.2)	5.1 (0.6)
HbF (%)				
Range	6–10[a]	0	0	7–14[b]
Positive osmotic fragility test				
No. (%)	73 (81.1)	10 (83.3)	198 (63.9)	81 (81.0)

[a] *3 cases.*
[b] *2 cases.*
Group 1 = thalassaemia carriers; Group 2 = indeterminate; Group 3 = iron deficient; Group 4 = obligatory carriers (parents of known thalassaemia patients).
SD = standard deviation.
Hb = haemoglobin; Hct = haematocrit; RBCs = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; TIBC = total iron binding capacity; TS = transferrin saturation.

transferrin saturation, low serum iron and normal to high TIBC (Table 1).

- We also included 100 obligatory carriers, Group 4, as controls. They had high levels of HbA2 (> 3.6%) and normal levels of iron parameters (Table 1). High HbF levels were found in 2 cases only.

The rate for positive osmotic fragility test was highest, 83.3%, for Group 2, closely followed by Group 1 and Group 4, both > 80%. The lowest rate, 63.9%, was for Group 3, the iron deficiency group (Table 1).

There were no major differences between Group 1, the β-thalassaemia carrier group, and Group 4, the obligatory carrier group, in any of the tests. Both groups showed microcytosis (MCV < 80 fL), hypochromia (MCH < 27 pg), high HbA2 (> 3.6%) and normal iron parameters (Table 1).

There was a significant correlation between degree of anaemia (Hb level) and degree of microcytosis in Group 1, the β-thalassaemia carrier group ($P < 0.01$; $r = 0.7$) and in Group 4, the obligatory carrier group ($P < 0.001$; $r = 0.3$).

In Group 3, the iron deficiency group, and Group 2, MCV was significantly correlated with Hb level ($P < 0.01$ and < 0.0001 respectively; $r = 0.7$ and 0.9 respectively). The MCV in these 2 groups was significantly correlated with the degree of iron deficiency. In Group 2, the MCV was statistically significantly correlated with serum iron and transferrin saturation ($P < 0.002$ and < 0.001 respectively; $r = 0.8$ for both); the correlation was also significant in Group 3 ($P < 0.0001$ for both; $r = 0.6$ for both).

For the one-tube osmotic fragility test for detection of β-thalassaemia carriers, sensitivity was 87.0%, specificity 34.1%, positive predictive value 47.2%, negative predictive value 82.3% and overall accuracy 53.0%.

Discussion

In this study, microcytosis was significantly correlated with the degree of anaemia in the screened β-thalassaemia carrier group but correlation between the MCV and iron parameters was not statistically significant. In the iron deficiency group, microcytosis was significantly correlated with the degree of anaemia as well as the degree of iron deficiency. It has previously been reported that microcytosis and hypochromia in thalassaemia trait may be greater than expected for the mild degree of anaemia, but in iron deficiency cases, microcytosis was related to the degree of anaemia [*13*].

Our 412 cases with microcytosis were subdivided into 3 groups according to their HbA2 levels. Group 1 had high HbA2, 3.9%–6.0%; Group 2 had borderline HbA2, 3.3%–3.5%; Group 3 had low to normal HbA2, 1.3%–2.4%. In previous reports, many researchers considered HbA2 levels 3.8%–8.0% indicative of β-thalassaemia trait and 3.3%–3.8 % as borderline, requiring further assessment [*14*]. These values were in accordance with the HbA2 levels of our obligatory carriers, Group 4.

In our study, elevated HbF was detected in 3.3% of cases in Group 1. This is considerably fewer than the 30%–50% of cases with high HbF (> 1.0%) that have been reported previously [*9*]. It was, however, in keeping with the levels in the obligatory carriers in Group 4, where 2% only had elevated HbF levels.

Iron parameters were normal in the 2 carrier groups, but abnormal levels were found in the other 2 groups. Normal serum iron levels range from 70 µg/dL to 200 µg/dL. Low levels are seen in iron deficiency states, and high levels are found in ineffective erythropoiesis and iron overload. The TIBC has a normal range of 250–435 µg/dL, mean 320 µg/dL, and it is raised in iron deficiency [*15*].

In our study, transferrin saturation was used as an index of iron status, and values < 16% were taken as an indicator of iron deficiency, as seen in Group 2 and Group 3. Many other researchers have reported that transferrin saturation < 16% constitutes good evidence of iron deficiency only in conjunction with low MCV [*9–15*].

In Group 2, iron deficiency was indicated by the different index of iron status and borderline HbA2. It has been reported that such concordance results in reduction of HbA2 synthesis, and the HbA2 value may be reduced to borderline or even normal levels in β-thalassaemia trait, depending on the severity of the anaemia [*16*].

The osmotic fragility test was positive in 81.1% of the carrier group and 63.9% of the iron deficiency group. This is lower than that reported in a previous study, 96% in a thalassaemia carrier group and 80% in an iron deficiency group [*11*].

In our study the one-tube osmotic fragility test showed limitations as a screening test for β-thalassaemia. This has been reported in other studies. The test is potentially useful although it cannot replace automated red cell indices, and specificity would clearly be much worse in a population where iron deficiency is common [*17*]. On the other hand previous reports have found the one-tube osmotic fragility test could be used as an effective preliminary screening for identifying thalassaemia carriers [*7,18*].

Our study verified a high prevalence of iron deficiency status among the screened sample. This has been reported in other studies, iron deficiency remains the most common cause of microcytic anaemia worldwide [*19*].

In conclusion, the β-thalassaemia carrier rate in Egypt is not less than 9%, and 1.2% of those we tested would require further evaluation. The combination of MCV, HbA2 level by column chromatography and transferrin saturation seems useful for a thalassaemia screening programme in Egypt. The osmotic fragility test has limited value in our population, where iron deficiency is prevalent.

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