Osmotic fragility test in heterozygotes for α and β thalassaemia

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SUMMARY This study shows that the combination of heterozygous β thalassaemia and deletion heterozygous ($-\alpha/\alpha$) or homozygous ($-\alpha/-\alpha$) α^+ thalassaemia may result in the production of erythrocytes which have normal mean volume and haemoglobinisation but decreased osmotic fragility. Based on this finding and previous studies, which have shown that β thalassaemia screening by the osmotic fragility test may miss a significant proportion of β thalassaemia heterozygotes, we conclude that β thalassaemia screening in a population in which both α and β thalassaemia are prevalent should combine the one tube osmotic fragility test with electronic measurement of red blood cell indices in the initial screening process.

Microcytosis and reduced Hb content per cell are typical features of the red blood cells in β thalassaemia heterozygotes, which have been widely used for population screening.¹ However, it has recently been shown that the combination of heterozygous β thalassaemia with deletion heterozygous ($-\alpha/\alpha$) or homozygous ($-\alpha/-\alpha$) α^+ thalassaemia produces larger and more completely haemoglobinised red blood cells as compared to those of β thalassaemia heterozygotes with a full complement of four α globin structural genes ($\alpha\alpha/\alpha\alpha$).² Among these double heterozygotes, a minority have been found to have red blood cell indices within the normal range.³ In populations in which both α and β thalassaemia are common, screening for β thalassaemia based on MCV/MCH values may, therefore, miss a significant proportion of heterozygotes. In Sardinians, for example, 3% of β thalassaemia carriers would have been missed with a screening strategy based on electronic measurement of MCV as the initial screening process.³

With the osmotic fragility test, largely used in the past in population screening for β thalassaemia, a low proportion of false negatives has been detected.^{4,5} It seemed worthwhile, therefore, to study the osmotic fragility in those β thalassaemia heterozygotes in whom the coinheritance of α thalassaemia produced normal red blood cell indices.

Material and methods

In this study we included 24 Sardinian β thalas-

saemia heterozygotes who were selected on the basis of normal red blood cell indices and whose only distinguishing feature was an increase in Hb A₂ levels. All of them had balanced or reduced α/β globin chain synthesis ratio. α globin gene mapping showed in 10 the deletion of a single α globin structural gene ($-\alpha/\alpha$) (rightward deletion) and in 14 the deletion of two α globin genes, one on each chromosome ($-\alpha/-\alpha$). The molecular defect of the β globin gene in all of them was the nonsense mutation (CAG→TAG) at the codon corresponding to amino acid 39 (β^0 39). Analysis of red blood cell indices and globin chain synthesis and α globin gene mapping in 10 of these heterozygotes have already been reported.³

Red blood cell indices, Hb A₂ and Hb F quantification, G6PD activity, and globin chain synthesis analysis in peripheral blood reticulocytes were performed as previously described.³ α globin gene mapping was carried out by digestion of the DNA with the restriction endonucleases BamHI and BglII and hybridisation with an α or ξ globin specific probe respectively by previously described methods.³ Specific diagnosis of the β thalassaemia mutation was accomplished by the oligonucleotide method.⁶

Red cell osmotic fragility was determined either with the classical multiple tube technique using fresh blood as well as blood incubated for 24 hours at 37°C⁷ or with the one tube method according to Malamos *et al.*⁴ The one tube method was carried out with 0.4% buffered NaCl, and 20 μ l of blood was pipetted into 5 ml of the saline solution and thoroughly mixed. After 30 to 45 minutes the tubes were inspected. The test was considered abnormal if

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the sample appeared cloudy or had a sediment of intact cells or both.

Results

The table summarises the haematological characteristics of the heterozygotes for α and β thalassaemia included in this study in relation to the complement of α globin genes. In all these cases the osmotic fragility, either using the classical method (figure) or the one tube system, was decreased. This decrease became more marked after sterile incubation for 24 hours (figure).

Discussion

This study shows that the combination of heterozygous β^0 thalassaemia and deletion heterozygous ($-\alpha/\alpha$) or homozygous ($-\alpha/-\alpha$) α^+ thalassaemia may result in the production of erythrocytes which have normal mean volume and haemoglobinisation, but decreased osmotic fragility. Since a decrease in osmotic fragility is the result of an excess of surface area relative to the volume, regardless of the absolute value of either parameter, it seems that in β thalassaemia heterozygotes the coinheritance of α thalassaemia, while tending to normalise the volume and the haemoglobinisation of red blood cells, may leave unaffected or produce a further increase of the surface area, at least in a subpopulation of red blood cells. This may explain the persistent abnormality of osmotic fragility in spite of the normal volume and haemoglobin content per cell.

These heterozygotes for α and β thalassaemia would have been missed in a carrier screening based on the electronic measurement of MCV/MCH, but would have been detected with a screening process based on the osmotic fragility test as an initial screening test. On the other hand, screening with the osmotic fragility test alone in the initial testing may miss approximately 3% of β thalassaemia carriers.⁵ The most likely explanation for these false negatives is the association of inherited or acquired conditions which may reduce the ratio between the surface area and the volume, typical of the red blood cells from β thalassaemia heterozygotes. The excess surface area may be reduced by an insufficient supply of ATP for acylation of the membrane lipids, which could be related to many different acquired or genetic conditions, and the volume may be increased for associated conditions, such as G6PD deficiency or pregnancy, or may result from the intake of oral contraceptives, alcohol, and several other drugs.

Based on these findings, we may conclude that in populations in which both α and β thalassaemia are

TABLE Haematological characteristics of heterozygotes for α and β thalassaemia in relation to the α globin genotype*.

α globin genotype	Sex	No	RBC ($\times 10^{12}/l$)	Hb (g/dl)	MCV (fl)	MCH (pg)	Hb A ₂ (%)	Hb F (%)	α/β ratio
$-\alpha/\alpha$	M	5	5.30 $\pm$ 0.80	14.14 $\pm$ 1.48	81 $\pm$ 3.6	26.9 $\pm$ 2.38	5.01 $\pm$ 1.12	1.11 $\pm$ 1.84	0.95 $\pm$ 0.52
	F	5	5.18 $\pm$ 0.48	13.44 $\pm$ 0.80	80 $\pm$ 6.6	25.8 $\pm$ 1.72	5.08 $\pm$ 0.74	0.96 $\pm$ 0.70	0.76-0.91-0.89†
$-\alpha/-\alpha$	M	9	5.47 $\pm$ 0.78	14.97 $\pm$ 2.28	82 $\pm$ 8.3	27.5 $\pm$ 2.80	5.15 $\pm$ 0.82	0.65 $\pm$ 0.32	0.86 $\pm$ 0.16
	F	5	4.63 $\pm$ 0.90	12.04 $\pm$ 2.02	78 $\pm$ 1.7	26.5 $\pm$ 1.96	5.09 $\pm$ 0.48	0.64 $\pm$ 0.12	0.91-0.75†

*The red blood cell indices, α/β globin chain synthesis ratios, and α globin gene mapping of 10 of these heterozygotes have already been reported in a separate paper.³

†Values available only for three and two subjects respectively.

FIGURE Osmotic fragility of fresh (a) and incubated (b) red blood cells from a representative group (12 subjects) of β^0 thalassaemia carriers with a deletion of one or two α globin structural genes.

common, the one tube osmotic fragility test should be used in association with the electronic measurement of red blood cell indices in the initial screening process. Alternatively, testing for Hb A₂ should be incorporated into the initial set of tests, as we have already suggested.⁸

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