Haematological characteristics of the β^0 thalassaemia trait in Sardinian children

R GALANELLO, S DE VIRGILIIS, M ADDIS, E PAGLIETTI, R RUGGERI, AND A CAO

From the 2nd Paediatric Clinic, University of Cagliari, Sardinia, Italy

SUMMARY We report red cell indices and haemoglobin (Hb) A₂ levels in Sardinian children with heterozygous β^0 -thalassaemia and in normal controls aged 6 months to 12 years. Iron-deficient children and those with haematological findings indicative of α -thalassaemia were excluded. As in adult carriers, these subjects have significantly increased mean red cell counts and significantly reduced mean Hb levels, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), haematocrit, and mean corpuscular haemoglobin concentration. From 66 to 76% of the heterozygous β^0 thalassaemia children examined were anaemic. MCH and MCV were within the normal range in 2.8% of these children. Serum ferritin levels showed no difference from those of normal controls.

Adult β -thalassaemia heterozygotes are known to have the following haematological characteristics: anisopoikilocytosis, decreased osmotic fragility, increased mean red cell count, reduced haemoglobin (Hb), mean corpuscular volume (MVC), and mean haemoglobin concentration (MCH), increased Hb A₂ levels, and a slight inconstant increase of Hb F.^{1,2}

Slight differences in these manifestations have been found between different racial groups, depending on genetic heterogeneity and environmental influences.^{1,3-7}

However, the haematological characteristics of β -thalassaemia trait have not been studied extensively in children. Detailed information on this point is needed, firstly, to establish the criteria for suspecting β -thalassaemia trait from red cell indices analysis at this age and, secondly, to determine the range of variability of these characteristics. In particular, knowledge of this range would make it easier to detect a superimposed anaemia in a child with β -thalassaemia trait. The Sardinian population offers a unique opportunity to carry out this study, since there is a high β -thalassaemia carrier rate (12.9%), and β -thalassaemia is almost exclusively of the β^0 type.^{7,8}

We present the results of a survey of red cell indices and Hb A₂ determination in Sardinian children with β -thalassaemia trait.

Subjects

One hundred and forty β -thalassaemia heterozygous children aged 6 months to 12 years (85 boys and 55 girls), and 126 normal controls of the same age (71 boys and 55 girls), were studied. The subjects were divided into the following age groups according to Dallmann:⁹ 0.5-2, 2-6, and 6-12 years (Table 1).

CRITERIA OF DIAGNOSIS

Diagnosis of β -thalassaemia trait was based on increased Hb A₂ levels (> 4%) associated with microcytosis and normal serum iron. Children who might have iron deficiency according to the following criteria—percent transferrin saturation (calculated from the ratio serum iron/total iron binding capacity) lower than 10,⁹ and/or serum ferritin below 10 μ g/l,¹⁰ were excluded. Since there is a high incidence of α -thalassaemia (about 12% of newborns with Hb Bart's levels > 1%) in our population (unpublished results), children with normal Hb A₂ levels, normal serum iron, and MCV lower than normal for age (70 fl for 0.5-2, 74 fl for 2-6, and 76 fl for 6-12 years)⁹ were excluded.

Methods

Venous blood was drawn in all subjects between 0800 and 1000 after an overnight fast and collected in part in EDTA and in part in an iron-free tube.

Received for publication 28 February 1980

Table 1 Red cell indices and Hb A₂ levels in heterozygous β^0 -thalassaemia (HBT) children (mean $\pm$ 1 SD and range)

Age (yr)	Subject	No.	RBC ($\times 10^{12}/l$)	Hb (g/dl)	MCV (fl)	Hct %	MCH (pg)	MCHC (g/dl)	Hb A ₂ %	Transferrin saturation %
0.5-2	Normal	20	4.8 $\pm$ 0.4 4.35-5.80	12.7 $\pm$ 0.9 11.20-14.20	75.6 $\pm$ 4.0 70.0-83.0	36.0 $\pm$ 2.4 31.7-40.8	26.4 $\pm$ 2.3 21.9-29.8	35.1 $\pm$ 1.7 31.10-38.0	2.5 $\pm$ 0.3 1.9-3.0	23.1 $\pm$ 6.3 12.3-31.1
	HBT	27	5.6 $\pm$ 0.5 4.5-6.3	10.7 $\pm$ 0.8 9.0-12.5	59.9 $\pm$ 3.1 55.0-66.0	32.7 $\pm$ 2.9 24.0-37.7	19.1 $\pm$ 1.7 15.7-26.0	32.6 $\pm$ 3.4 28.2-47.3	5.1 $\pm$ 0.5 4.1-6.0	22.1 $\pm$ 8.3 12.0-36.7
2-6	Normal	56	4.7 $\pm$ 0.4 3.9-5.8	12.4 $\pm$ 0.7 10.8-13.8	78.1 $\pm$ 2.9 74.0-85.0	36.6 $\pm$ 2.1 31.6-42.6	26.3 $\pm$ 1.6 23.0-30.0	34.0 $\pm$ 1.6 30.0-36.9	2.5 $\pm$ 0.3 1.5-3.0	25.4 $\pm$ 8.9 12.3-47.6
	HBT	50	5.6 $\pm$ 0.4 4.7-6.5	10.7 $\pm$ 1.0 8.9-13.6	61.2 $\pm$ 3.5 55.0-74.0	33.7 $\pm$ 2.6 28.1-41.1	19.0 $\pm$ 1.6 14.4-22.8	31.7 $\pm$ 2.3 26.2-38.6	5.3 $\pm$ 0.6 4.1-6.9	28.0 $\pm$ 8.9 11.2-40.4
6-12	Normal	50	4.8 $\pm$ 0.4 3.5-5.4	13.1 $\pm$ 0.8 11.2-15.8	79.8 $\pm$ 2.5 76.0-86.0	38.3 $\pm$ 2.5 29.8-43.0	27.1 $\pm$ 1.7 23.4-30.6	34.2 $\pm$ 1.5 31.0-37.7	2.6 $\pm$ 0.2 2.2-3.0	29.0 $\pm$ 10.1 13.5-52.2
	HBT	63	5.7 $\pm$ 0.4 4.7-6.5	11.04 $\pm$ 1.0 8.8-13.6	61.6 $\pm$ 4.6 56.0-79.0	34.3 $\pm$ 2.6 27.0-40.9	19.3 $\pm$ 1.9 16.0-26.2	32.1 $\pm$ 1.4 29.0-35.1	5.2 $\pm$ 0.6 4.1-6.6	27.2 $\pm$ 7.8 12.5-46.4

Red blood cell count, MCV, and haematocrit were determined on a Coulter model ZBI, daily standardised with commercial standard 4C. Haemoglobin levels were determined on a Coulter haemoglobinometer. Serum iron and total iron-binding capacity were measured by Lauber's method.¹¹ Serum ferritin was determined by a commercially available RIA-kit (Ramco Laboratories Inc, Houston, Texas, USA). Hb A₂ was quantitated by DE-52 microchromatography according to Huisman *et al.*¹²

Results

Since there were no differences between males and females the results obtained were not divided according to sex.

As can be seen in Table 1, heterozygous β -thalassaemia children in all age groups considered have significantly increased mean red cell counts ($P < 0.001$) and significantly reduced Hb levels ($P < 0.001$), MCV ($P < 0.001$), MCH ($P < 0.001$), haematocrit ($P < 0.001$), and mean corpuscular haemoglobin concentration ($P < 0.001$). There were no differences in reticulocyte counts. These differences between β -thalassaemia heterozygous children and controls may be superimposed on those already found in our adult groups.⁷ Taking 2 standard deviations below the normal haemoglobin mean as the normal lower limit, 66-76% of the heterozygous β -thalassaemia children examined were considered to be anaemic (Table 2).

Defining microcytosis as MCV less than 2 SD below normal mean values (67.6 fl for 0.5-2, 72.3 fl for 2-6, and 74.8 fl for 6-12 years), 2.8% of β -thalassaemia heterozygotes had normal values.

Using similar criteria for MCH (21.9 pg for 0.5-2, 23.1 pg for 2-6, and 23.7 pg for 6-12 years), 2.8% of β -thalassaemia heterozygotes also had normal values.

The median value of serum ferritin concentration for 30 β -thalassaemia children was 31.5 ng/l (95% range 10-100 ng/l) and was not different from that of

Table 2 Percentage of heterozygous β^0 -thalassaemia children with anaemia*

Age (yr)	Hb (g/dl)*	% Anaemic
0.5- 2	10.9	66.7
2 - 6	11.0	66.0
6 -12	11.5	76.2

*Hb (g/dl) < mean minus 2 SD.

normal controls (30.6 ng/l, 95% range 9-101 ng/l, in 28 subjects).

Discussion

In this study the haematological characteristics of heterozygous β -thalassaemia in children aged 6 months to 12 years were found to be similar to those already seen in adults. However, considering normal mean Hb levels minus 2 SD as the lower normal limit in children as in adults, β -thalassaemia heterozygous children showed a higher incidence of anaemia (66-76%) than heterozygous adults (about 40%) of the same population. The likely explanation of this finding may be a minor compensation of the β -chain synthesis defects in heterozygous β -thalassaemia children with anaemia. This may depend on a relatively minor erythroid hyperplasia and/or a lower β -chain output from the normal β -chain gene. Both the MCV and MCH were normal, ie, higher than mean minus 2 SD, in 2.8% of β -thalassaemia heterozygous children. Therefore, in children as in adults,⁷ the use of MCV or MCH for β -thalassaemia screening will give some false negatives.

The results of this study have practical implications in the identification of β -thalassaemia trait and in the diagnosis of hypochromic microcytic anaemia in children.

Information on the haematological indices ranges of heterozygous β -thalassaemia children is particularly useful in the identification of superimposed anaemia in these subjects. However, it should be

pointed out that since there are some variations in haematological indices of adult heterozygous β -thalassaemia in different racial groups,³⁻⁷ the range of variability in children must be assessed in the various populations.

This work was supported by grants from Assessorato Igiene e Sanità Regione Autonoma della Sardegna, Sindacato Lavoratori Bancari, NIH grant No. 1 RO1 HL 24173-01, and CNR No. 79.03347.86.

References

- ¹ Weatherall DJ, Clegg JB. *The Thalassaemia Syndromes*. 2nd ed. Oxford: Blackwell Scientific Publications, 1972.
- ² Piomelli S, Siniscalco M. The hematological effects of glucose-6-phosphate dehydrogenase deficiency and thalassemia trait: interaction between the two genes at the phenotype levels. *Br J Haematol* 1969;**16**:537-49.
- ³ Knox-Macaulay HHM, Weatherall DJ, Clegg JB, Pembrey ME. Thalassaemia in the British. *Br Med J* 1973;**3**:150-5.
- ⁴ Pootrakul P, Wasi P, Na-Nakorn S. Haematological data in 312 cases of β -thalassaemia trait in Thailand. *Br J Haematol* 1973;**24**:703-12.
- ⁵ Pootrakul P, Assawamunkong S, Na-Nakorn S. β^+ thalassemia trait: hematological and hemoglobin synthesis studies. *Hemoglobin* 1976-77;**1**:75-83.
- ⁶ Millard DP, Mason K, Serjeant BE, Serjeant GR. Comparison of haematological features of the β^0 and β^+ thalassemia traits in Jamaican Negroes. *Br J Haematol* 1977;**36**:161-70.
- ⁷ Galanello R, Melis MA, Ruggeri R, et al. β^0 thalassemia trait in Sardinia. *Hemoglobin* 1979;**3**:33-46.
- ⁸ Cao A, Galanello R, Muroli PP, et al. Thalassaemia types and their incidence in Sardinia. *J Med Genet* 1978;**15**:443-7.
- ⁹ Dallmann PR. New approaches to screening for iron deficiency. *J Pediatr* 1977;**90**:678-80.
- ¹⁰ Siimes MA, Addiego JE, Dallmann PR. Ferritin in serum: diagnosis of iron deficiency and iron overload in infants and children. *Blood* 1974;**43**:581-90.
- ¹¹ Lauber K. Determination of serum iron and iron binding capacity without deproteinization. *Z Klin Chem* 1965;**3**:96-9.
- ¹² Huisman THJ, Schroeder WA, Brodie AN, Mayson SM, Jakway J. Microchromatography of haemoglobins. III A simplified procedure for the determination of haemoglobin A₂. *J Lab Clin Med* 1975;**86**:700-2.

Requests for reprints to: Professor Antonio Cao, Clinica Pediatrica II, Università degli Studi di Cagliari, Via Porcell, 1, 09100 Cagliari, Sardinia, Italy.