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SHORT COMMUNICATION

Hematological phenotype and the type of β thalassemia mutation in Brazil

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ABSTRACT

The type of β thalassemia mutation found in heterozygous individuals is believed to influence hematological symptoms. Our data demonstrated that patients with the mild mutation (β IVSI-nt 6) have a higher mean corpuscular hemoglobin (MCH) than those with the severe forms (β^o39 or β IVSI-nt 1), and the hemoglobin A_2 levels were lower in β^o mutations than in the β IVSI-nt 6 mutation. However, in contrast to previous studies, we were not able to indicate MCH as a clear discriminator between the β^o and β^+ mutations.

INTRODUCTION

β thalassemia in the heterozygous state presents a typical hematological picture characterized by microcytosis, reduced red blood cell hemoglobin concentration and high hemoglobin A_2 (Hb A_2) levels in Hb electrophoresis. The severity of these parameters varies from clearly abnormal to close to normal. These variations are related to several factors which include co-inherited α thalassemia and associated iron deficiency. A study carried out in Israel has suggested that the degree of reduction in MCH (mean corpuscular hemoglobin) or MCV (mean corpuscular volume) indicates the type of β thalassemia mutation (Rund *et al.*, 1992).

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MATERIAL AND METHODS

Seventy β thalassemia heterozygotes ranging from 16 to 66 years old were studied. The β thalassemia mutation and the α globin arrangement were defined for each individual. Serum iron was evaluated in all patients using a serum iron assay kit (assay without deproteinization - Boehringer Mannheim Gmblt) and a ferritin kit (Ferritin Fluorometric Enzyme Immunoassay-Baxter Shatus, USA) to determine possible iron deficiency. Blood samples were collected in EDTA. Red blood cell counts were made with a model SSr Coulter Counter. Hemoglobin A_2 (Hb A_2) was determined spectrophotometrically after electrophoretic separation on cellulose acetate using TEB buffer, pH 8.6. Hb F was determined by alkali denaturation (Betke *et al.*, 1959). DNA was isolated from peripheral blood leukocytes. The alfa globin arrangement was investigated by Southern blot analysis, as previously described (Sonati *et al.*, 1991).

β thalassemia mutations were identified by hybridizing PCR amplified DNA with ^{32}P labelled synthetic oligonucleotide probes. The primers for amplifi-

Table I - Hematological features and electrophoretic data (mean ± SD) for β thalassemia heterozygotes in relation to the type of β thalassemia mutation.

Mutation	N	RBC $10^6/mm^3$	Hb g/dl	Ht %	MCV fl	MCH pg	Hb A_2 %	Hb F %
β^o39	45	5.4 ± 0.8	11.2 ± 1.4	35.6 ± 3.7	66.1 ± 8.2	20.7 ± 2.4	5.0 ± 0.8	1.6 ± 1.3
β IVSI-110*	14	5.0 ± 0.7	10.8 ± 2.3	33.4 ± 5.7	69.0 ± 9.3	21.5 ± 3.8	4.9 ± 0.8	0.8 ± 0.4
β IVSI-6*	5	5.6 ± 0.4	13.3 ± 1.1	41.3 ± 4.4	72.4 ± 7.0	23.3 ± 0.6	3.6 ± 0.6	1.0 ± 0.6
β IVSI-1*	4	5.4 ± 0.5	10.6 ± 0.3	33.8 ± 0.3	63.7 ± 6.7	20.3 ± 2.3	5.7 ± 1.7	1.1 ± 0.3
Others*	2	6.1 ± 0.2	11.5 ± 0.4	39.0 ± 3.4	63.3 ± 2.3	19.2 ± 0.2	5.1 ± 0.1	1.3 ± 0.4

*β IVSI-110 = β IVSI-nt 110; β IVSI-6 = β IVSI-nt 6; β IVSI-1 = β IVSI-nt 1; fl = fentoliters.
Others = The mutation was not identified.

cation, the probe sequences, and the dot blot hybridization procedures have been described elsewhere (Costa *et al.*, 1990; Martins *et al.*, 1993). The statistical analysis was performed using the non-parametric Kruskal-Wallis test.

RESULTS

The predominant mutations in the Brazilian population are the codon 39 (C → G) nonsense mutation and the β IVSI-nt 110, β IVSI-nt 6 and β IVSI-nt 1 mutations (Costa *et al.*, 1990; Martins *et al.*, 1993). Table I shows the comparison between the hematological and electrophoretic data and the type of β thal mutation. The heterozygotes for the β IVSI-nt 6 mutation had significantly higher MCH and Hb A_2 values when compared to the heterozygotes for β^o39 and β IVSI-nt 1.

The hemoglobin levels and MCV were also higher among the β IVSI-nt 6 heterozygotes than in the other mutations, although the differences were not significant (Table II). No case of deletional type α thalassemia was detected and the iron status was normal in the heterozygotes.

DISCUSSION

Most of the β thalassemia cases in Brazil occur in individuals of Mediterranean origin (Martins *et al.*, 1993). In the present study, common conditions such as α thal or iron deficiency, which may interfere with the red cell indices, were excluded.

Our data demonstrate that the mild mutations such as β IVSI-nt 6 have a higher MCH than the severe forms, such as β^o39 or β IVSI-nt 1, and are in agreement with the report of Rund *et al.* (1992) for populations from Israel and with Rosatelli *et al.* (1992) for β thalassemia heterozygotes from Italy. These data are very probably

Table II - Statistical comparison (Kruskal-Wallis test) among the bearers of different mutations for β thalassemia, Hb A_2, Hb F, MCV and MCH.

	Prob. A_2	Prob. F	Prob. MCV	Prob. MCH
Total	0.11	0.17	0.39	0.10
β^o39/β^+110*	0.69	0.02	0.41	0.30
β^o39/β^+6*	0.01	0.56	0.13	0.02
β^o39/β^o1*	0.84	0.77	0.72	0.79
β^+110/β^+6*	0.52	0.47	0.41	0.10
β^+6/β^o1*	0.04	0.82	0.19	0.04
β^o/β^+	0.14	0.03	0.14	0.05

Prob. = Probability.
*β^+110 = β IVSI-nt 110; β^+6 = β IVSI-nt 6; β^o1 = β IVSI-nt 1.

associated with the variable degree of residual output of β globin chains from the β^+ locus, compared to an absence of production by the β^o locus. The MCH and MCV values have similar distributions. Rund *et al.* (1992) suggested that such values could be used in the identification of different mutation types. However, in our study, they were not useful in discriminating different mutations or for establishing differences between β^+ and β^o, since there was a significant overlap in the values obtained (Figure 1).

Some individuals with the mild mutation β IVSI-nt 6 have values close to normal, which suggests that they may be missed by carrier screening based only on MCV or MCH determination. Our data support those who advocate the quantification of Hb A_2 in the first set of examinations for the detection of β thalassemia carriers, in agreement with Rosatelli *et al.* (1992).

The observation that the Hb A_2 levels are lower in β^o mutations than in mild β^+ mutations supports previous data which suggest that the presence of the β chain in β^+ mutations limits the posttranslational increased assembly of Hb A_2, when compared to β^o mutations (Bunn and Forget, 1986).

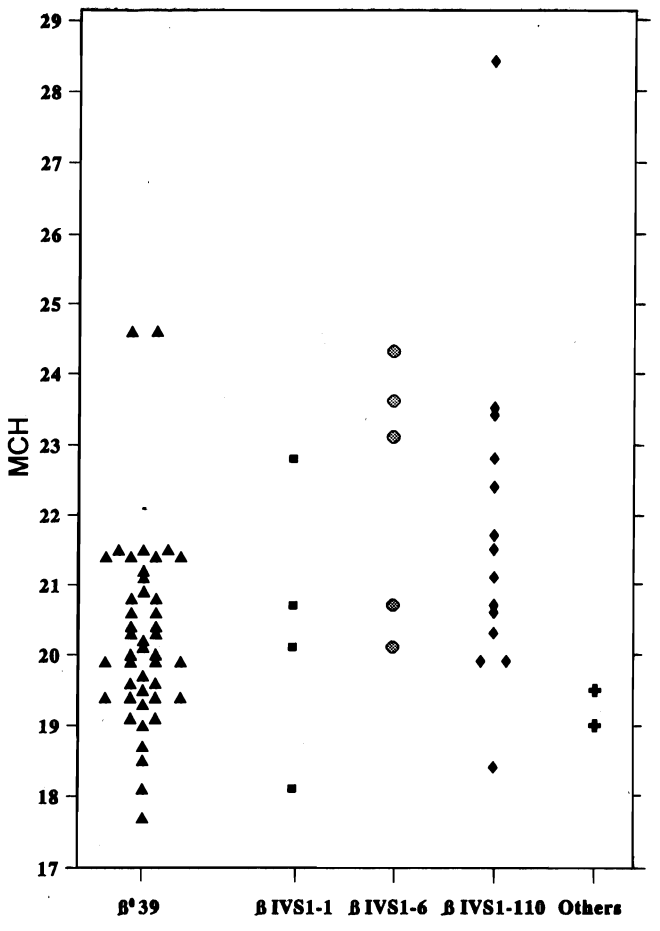

Figure 1 - The relationship between mean corpuscular hemoglobin (MCH) and the mutation in β thalassemia heterozygotes. Each form represents an individual carrying one of the four mutations. Note that there is no clear cut-point for MCH which could be useful in discriminating between the different mutations.

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RESUMO

O heterozigoto para a talassemia β apresenta um típico padrão hematológico. A severidade desses parâmetros varia consideravelmente de valores claramente anormais até próximos à normalidade. Postula-se que essa variação seria influenciada pelo tipo de mutação de talassemia beta encontrada e que portanto seria possível suspeitar-se do tipo de alteração pelos índices hematológicos. Nossos resultados demonstram que mutações brandas (β IVSI-nt 6) mostram maiores níveis de hemoglobina corpuscular média (MCH) do que as formas mais severas (β^o39 or β IVSI-nt 1) e que os níveis de hemoglobina A_2 são menores em mutações β^o do que em formas brandas (β IVSI-nt 6). Entretanto, contrariando estudos anteriores, não pudemos indicar o MCH como um discriminador entre as mutações β^o ou β^+.

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