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FEASIBILITY OF MOLECULAR DIAGNOSIS OF α-THALASSEMIA IN THE EVALUATION OF MICROCYTOSIS

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ABSTRACT

Microcytosis is a common hematological finding, usually related to iron deficiency or β-thalassemia. When both of these conditions are excluded, α-thalassemia must be considered in the differential diagnosis. No simple biochemical test is able to diagnose the α-thalassemia trait. Using PCR amplification of the breakpoint in deletional forms, and amplification of the α_2 gene and restriction enzyme digestion in non-deletional forms, we identified the α-thalassemia carrier status in 42 out of 51 (82%) patients with microcytosis or slight microcytic anemia, unrelated to iron deficiency or β-thalassemia. Our results underline the usefulness of molecular tests in clinical practice.

Key words: a-thalassemia, microcytosis, PCR

Microcytic anemia is a common hematological abnormality, usually resulting from iron deficiency or β-thalassemia.

Although α-thalassemia (for review see refs. #1 and #2) is frequent in Italy,[3-5] detailed data on its incidence throughout the country are not available. No simple biochemical test is able to diagnose α-thalassemia carriers, who have one or two α genes inactivated. The reduced α-chain availability decreases both hemoglobin A and A_2, leaving their respective proportions unchanged at hemoglobin electrophoresis. Globin chain synthesis studies are both expensive and time-consuming, and cannot provide the correct diagnosis in a significant proportion of cases, due to overlapping α/non-α ratios between normal and α-thalassemia carriers.[1,2]

Knowledge about the molecular basis of a-thalassemia allows for the specific analysis of the molecular defects. A series of tests are available in order to amplify the breakpoints in deletional forms,[2,6,7] and the ability of selectively amplifying both α_2 and α_1 genes provides the opportunity of studying non-deletional forms.[2] Investigating five molecular defects, we diagnosed α-thalassemia in 42/51 (82%) consecutive patients referred for microcytosis or mild microcytic anemia, unrelated both to β-thalassemia and iron deficiency.

Patients and Methods

Fifty-one subjects (23 males and 28 females) were included in the study. Nineteen were referred for mild microcytic anemia (Hb < 12 g/dL in females and <13 g/dL in males) and 32 for the isolated finding of microcytosis. MCV was < 80 fL and MCH < 26 µg in all cases. Hb A_2, serum iron, transferrin saturation and serum ferritin were determined by standard methods and were normal in all the cases.

DNA was prepared from buffy coats. PCR amplification was performed as described,[2] using suitable primers.[2,6,7] Restriction enzyme digestion of the PCR products was effected according to manufacturer recommendations.

Results

PCR assays using primers C2-C10[2] produced 2.1 Kb fragments in normal cases and 1.9 Kb fragments in the presence of $-\alpha^{3.7}$ deletion. Similarly, specific primers identified the breakpoint of --Med and $-\alpha^{20.5}$.[6] To test for the T→C substitution at the initiation codon and for the pentanucleotide deletion in intron I of the α_2 gene, a PCR product obtained using primers C3 and C10[2] was digested with Nco I or Hph I enzymes, respectively.

Table 1 shows the genotypes observed in the series examined. As expected, $-\alpha^{3.7}$ was the most common form, with nineteen heterozygotes and five homozygotes. Next we found the α_2 T→C substitution. Both --Med and the pentanucleotide deletion were rare, and $-\alpha^{20.5}$ was never observed in this series, but was detected only in cases of Hb H disease (not shown). Mean MCV was 76 fL (range

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Table 1. Frequency of the different α-thalassemia genotypes and alleles in the series examined.

Genotypes	*heterozygotes* N. (%)*		*homozygotes* N. (%)*	
– α 3.7	19	45	5	12
– – Med	3	7	–	
– 20.5	–		–	
Nco I	5	12	5	12
Hph I	4	10	1	2
Total n.	31		11	

Allele frequency in the series studied

– α 3.7		*– – Med*		*Nco I*		*Hph I*	
N.	%	N.	%	N.	%	N.	%
29	55	3	5.5	15	29	6	11

**% refers to the total number of patients.*

71.4-80 fL) and mean Hb was 13.6 (range 12.2-15.2 g/dL) in $-\alpha^{3.7}$ heterozygotes. No statistically significant difference was observed in Hb and MCV values in carriers of the different defects, which was probably due to the limited number of cases (not shown).

Discussion

The finding of microcytic red cells is a common problem in hematological practice. α-thalassemia is usually a presumptive diagnosis, after exclusion of β-thalassemia and iron deficiency. Although anemia is absent or unremarkable, it is important to diagnose α-thalassemia, in order to reassure patients and to avoid repeated and/or expensive analyses. Only the molecular approach allows for the precise diagnosis of α-thalassemia state. In screening five different mutations, we typed 82% of our patients. Other recent studies have used a similar approach to test patients with microcytosis,[8,9] reporting that a variable proportion of the cases analyzed is related to α-thalassemia. Careful selection of the samples increases the possibility of a correct diagnosis. However, in all series, a small proportion of patients remains undiagnosed. These cases may be explained by other undefined or unscreened forms of α-thalassemia. Family studies and globin chain synthesis may be useful in forms caused by unknown mutations. Some of the α-thalassemia defects reported in Italy[2,10] and not analyzed in this work are considered rare cases. However, PCR-based tests are available for ––Cal[5] and $-\alpha^{5.2}$.[10] It is likely that the number of undiagnosed cases will progressively decrease in the future as new forms of α-thalassemia are identified and became susceptible to molecular analysis.

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