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The Iron-Binding Capacity of Thalassemic Individuals

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Introduction

Thalassemia is characterized by several aberrations in the iron metabolism. In its " major " form the concentration of plasmatic iron is very high, conbined with the lack of an iron-binding capacity. In thalassemia " minor " Smith, Sisson, Floyd and Siegal (1950) observed variable results with respect to these two characteristics. The concentration of plasmatic iron was high in about half of the cases and the iron-binding component of the serum was fully saturated in 1/4 of the tested individuals; in 1/3 of the remaining the fraction saturated was also considerable. More than half of the results, therefore, were anomalous. As the above-mentioned authors emphasized, the confirmation of these data would supply an additional test to those already existing for the diagnosis of carriers of this disease. Therefore, we decided to carry on studies about this problem in a Caucasian family with several thalassemic individuals living in Pôrto Alegre, capital of the Brazilian State of Rio Grande do Sul.

Material and methods

The detection of thalassemia in the proposita IV-10 occurred during routine examinations made in the laboratory of one of us (O.G.H.). The examination was requested by the family doctor as a pre-operative measure. The tests made in this person and in members of her family were the following:

a) *Erythrocyte's morphology* – The blood samples were obtained by venipuncture. Immediately afterwards a blood smear was made which was stained according to the method of Rosenfeld (1947). Cytologic tests for the determination of sickling was also made with and without a reducing substance (sodium metabisulfite).

b) *Osmotic fragility and other hematological values* – The osmotic fragility was measured by the method of Todd and Sanford (1935). The dosage of hemoglobin was obtained after transforming it into cyanmethemoglobin and measuring it in a spectro-

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Ricevuto il 10. IX. 1964

Tab. 1. Clinical and hematological data obtained in members of the studied family

Pedigree no.	Sex	Age	Nationality of grand-parents	Osmotic fragility		Erythrocytes 10^6/mm^3	Hb	MCH	MCHC	MCV	Hemato-crit	Bilirubin mg%		Reticulo-cytes %	Leukocytes by mm^3	Target Cells	Iron treatment	Blood transfusion	Palpable spleen and liver
				Initial	Final							Total	Indirect						
Thalassemic individuals																			
II-12	♂	69	1	0.44	0.24	3.8	9.4	24.8	29.4	84.80	32	—	—	—	7,300	—	o	o	—
III-7	♂	47	2	0.42	0.26	7.7	11.8	15.2	25.6	59.60	46	1.7	1.0	2	5,700	+	o	o	o
IV-10	♀	19	3	0.44	0.26	6.3	9.2	14.6	24.9	58.50	37	0.8	0.5	3	7,400	+	*	o	o
IV-11	♂	18	3	0.42	0.24	7.6	10.8	14.4	25.7	55.20	42	1.4	0.9	2.5	8,100	+	**	o	o
Normal individuals																			
II-10	♀	68	4	0.44	0.34	4.4	11.4	28.2	30.0	87.20	38	—	—	—	5,200	—	o	o	—
II-13	♀	71	5	0.44	0.36	4.2	12.0	28.6	30.0	95.10	40	1.1	0.3	1.0	6,500	—	o	o	o
III-6	♀	44	6	0.42	0.34	4.9	12.0	25.0	28.6	85.40	42	1.0	0.6	0.5	9,600	—	o	o	o
IV-9	♂	20	3	0.44	0.34	5.2	12.6	24.6	28.6	84.30	44	—	—	—	5,800	—	o	o	o

* Occasional
** Periodic
O Observation made with negative results
— Not tested

1. Polish
2. Maternal grandparents Lithuanian; paternal Portuguese
3. Maternal grandparents Brazilian; paternal Lithuanian
4. Italian
5. Lithuanian
6. Maternal grandparents Italian; paternal Portuguese

https://doi.org/10.1017/S1120962300015523 Published online by Cambridge University Press

photometer Carl Zeiss PMQ II at 540 mu (Cartwright, 1958). The globular volume was measured as described in Wintrobe (1948). The dosage of bilirubin was performed by the method of Malloy and Evelyn (1937) and the reticulocytes counts as reported in Cartwright (1958).

c) *Serum iron content and latent iron-binding capacity* – The unsaturated or latent iron-binding capacity of the serum was determined according to the method of Rath and Finch (1949), the dosage of serum iron by the method of Kitzes, Elvehjem and Schuette (1944).

d) *Abnormal hemoglobins,* A_2 *component and fetal hemoglobin determinations* – The erythrocytes were washed three times with an isotonic solution of sodium chloride and hemolysed with the addition of an equal volume of distilled water and by freezing and thawing. The stroma was removed by centrifugation and the concentration was adjusted colorimetrically to give a concentration of 10 g by 100 ml. Electrophoretic analyses were made in vertical gel, in accordance with the technique described by Smithies (1959) using a 0.03 u borate buffer with pH 8.9 for the preparation of the gel. The concentration of fetal hemoglobin was determined by the technique of Singer, Chernoff and Singer (1951), three determinations were made from each sample.

e) *Blood groups* – Tests were made in relation to the ABO, MN and Rh systems. The tube test method was used and the antisera utilised were those manufactured by Johnson and Johnson and the Ortho Pharmaceutical Corporation.

Results and discussion

The studies performed are summarized in Tables 1, 2 and 3 and in Fig. 1. Table 1 shows the clinical and part of the hematological data. As is shown there not a single individual presented a palpable spleen or liver. None had received blood transfusions. IV-11 was submitted to periodic treatments with iron but IV-10 received iron treatment only twice. Three of the four tested thalassemic individuals showed a significant increase in the number of erythrocytes with a very low mean corpuscular hemoglobin; several target cells were also present. II-12, however, showed a normochromic anemia with a decrease in the number of erythrocytes and did not present target cells. In all of them the final hemolysis occurred in concentrations of sodium chloride quite below the normal limits. Reticulocyte counts were normal and the bilirubin concentrations did not show any significant increase.

Table 2 presents other hematological values observed in members of the studied family. The presence of abnormal hemoglobins was tested by electrophoresis in starch gel and by cytological examinations. In both tests the results were negative. Component A_2 was not increased, the same happening with the level of fetal hemoglobin. The allele which conditions thalassemia in this family, therefore, belongs to the group which does not cause the raise of A_2; its segregation pattern seems to be normal (Fig. 1). Blood groups were tested in order to detect possible cases of non-paternity. Not one exclusion was obtained with the antisera employed.

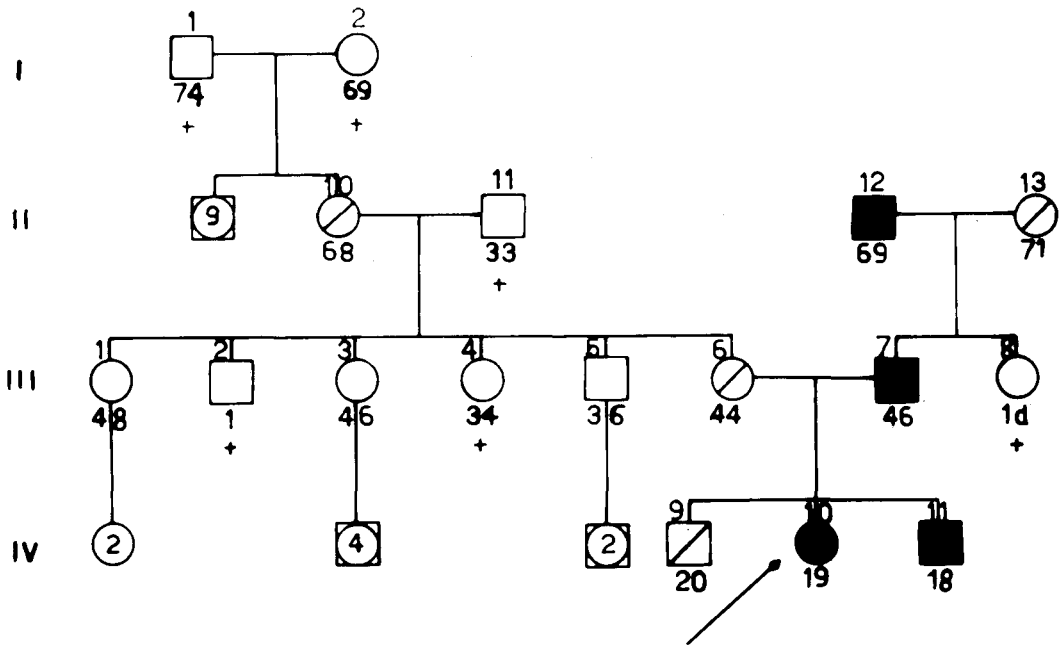

Fig. 1. Pedigree of the studied family. Circles and black squares = affected individuals; circles and squares with diagonal lines = studied, normal; + = dead; the arrow shows the proposita

Tab. 2. Other hematological values observed in members of the studied family

Pedigree	Hemoglobin types		Hb A_2	Hb F	Blood groups (Genotypes)		
	Starch gel	Cytological test			ABO	MN	Rh
Thalassemic individuals							
II-12	A	—	Normal	2.4	I^o I^o	L^M L^N	R^1 R^1
III-7	A	—	Normal	2.5	I^o I^o	L^M L^N	R^1 R^1
IV-10	A	—	Normal	3.9	I^o I^o	L^M L^N	R^1 R^1
IV-11	A	—	Normal	2.9	I^o I^o	L^M L^N	R^1 R^1
Normal individuals							
II-10	A	—	Normal	2.3	I^{A1} I^o	L^M L^M	r r
II-13	A	—	Normal	2.3	I^{A1} I^o	L^M L^N	R^1 R^1
III-6	A	—	Normal	1.5	I^o I^o	L^M L^M	R^1 r
IV-9	A	—	Normal	2.0	I^o I^o	L^M L^N	R^1 R^1

Tab. 3. Studies about the serum iron concentration and iron-binding capacity of the members of the studied family

Pedigree	Serum iron concentration (γ/100 ml)	Latent iron-binding capacity (γ/100 ml)	Total iron-binding capacity (γ/100 ml)	Saturation %
Thalassemic individuals				
II-12	190	550	740	26
III-7	90	200	290	31
IV-10	105	150	255	41
IV-11	120	250	370	32
Normal individuals				
II-13	180	550	730	25
III-6	100	200	300	33
IV-9	70	200	270	26

The serum iron-binding studies (Table 3) show that the amount of saturation is normal in the thalassemic individuals. The levels of serum iron are also within normal limits. Therefore our results do not confirm those of Smith et al. (1950). New studies are necessary to establish whether the variability in the obtained results is due to differences in the type of thalassemia present in the individuals of the two series or to technical problems. It seems evident, however, that studies of iron-binding capacity can help very little in the diagnosis of carriers of thalassemia.

Summary

A description is made of a Caucasian family of Lithuanian and Italian ancestry living in Pôrto Alegre, Brazil, in which there is segregation of the gene which conditions the type of thalassemia which does not raise the fraction A_2. The information obtained involves four generations in a total of thirty four individuals, eight of which were examined. Tests about the concentration of serum iron and the iron-binding capacity of thalassemic individuals resulted normal.

Acknowledgments

We want to express our gratitude to Dr. Casemiro V. Tondo for the determination of the levels of A_2 and fetal hemoglobin; to Miss Margarete V. Suñé for the blood group tests; and to Mr. Girley V. Simões for his assistance in the field work. In particular we want to express our thanks to Dr. Francisco M. Salzano for his valuable orientation and help in the preparation of this paper.

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RIASSUNTO

Si descrive una famiglia di razza bianca, con antenati di origine lituana e italiana, residente nella città di Pôrto Alegre, Brasile, nella quale si osserva la segregazione del gene che condiziona la talassemia, senza aumento della frazione A_2. Le informazioni ottenute riguardano quattro generazioni, per un totale di trentaquattro individui, otto dei quali sono stati esaminati. I test eseguiti sulla concentrazione del ferro sierico e sulla sua capacità di fissazione negli individui talassemici hanno dato risultati normali.

RÉSUMÉ

On fait la description d'une famille de race blanche, aux ancêtres lithuaniens et italiens, résidents à Pôrto Alegre, Brésil, dans laquelle on observe une ségrégation du gène qui conditionne l'apparition de la thalassémie sans augmentation de la fraction A_2. Les renseignements obtenus comprennent quatre générations, pour un total de trente-quatre individus, dont huit ont été examinés.

Des tests sur la concentration de fer du sérum sanguin et sur la capacité de fixation de fer chez les individus thalassémiques ont donné des résultats normaux.

ZUSAMMENFASSUNG

In der vorliegenden Arbeit wird ein Familie weisser Rasse beschrieben, deren Vorfahren litauischer und italienischer Abstammung sind und die in Pôrto Alegre, Brasilien, wohnen. In diesen ist das Gen, das Thalassämie hervorruft, ohne Erhöhung der Fraktion A_2 vorhanden. Wir haben Angaben über vier Generationen mit insgesamt 34 Individuen, von denen 8 untersucht wurden. Versuche über die Konzentration des im Blut enthaltenen Eisens an diesen thalassämischen Personen und Proben über die Einsenfixierung gaben normale Resultate.