Key words

Leptin, body fat, leukocyte count, hematological parameters

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Erythroid membrane protein defects in hereditary spherocytosis. A study of 62 Spanish cases

We studied the relative prevalences of erythroid cytoskeletal protein defects and their relationship with the clinical course of hereditary spherocytosis (HS) in 62 spanish patients (30 kindreds), 53 cases with familial history (21 kindreds).[1-5] Combined spectrin and ankyrin deficiency was the most prevalent abnormality, as previously described.[2,9,10]

Sir,

Clinical assessment, routine haemocytometry and denaturing electrophoresis (SDS-PAGE) in a continuous system of erythroid ghosts[6,7] with estimation of protein contents - spectrin/band 3 (Sp/B3) and ankyrin/band 3 (Ank/B3) ratios- were performed.

Table 1. Erythroid membrane protein contents and clinical course of hereditary spherocytosis.

HS		Sp/B3 Mean	Sp/B3 SD	Ank/B3 Mean	Ank/B3 SD	Ank/B3 corrected* Mean	Ank/B3 corrected* SD
	Control n=24	1.136 (1.043-1.242)	0.054	0.199 (0.197-0.203)	0.001	0.170 (0.161-0.177)	0.010
Mild	Propositi n=8	1.29 (0.973-1.130)	0.058	0.198 (0.195-0.202)	0.002	0.148 (0.087-0.171)	0.029
	Total n=20	1.032 (0.941-1.194)	0.066	0.197 (0.193-0.202)	0.002	0.154 (0.087-0.171)	0.021
Typical°	Propositi n=16 (s=4)	1.007 (0.934-1.155)	0.062	0.195 (0.188-0.206)	0.004	0.100 (0.014-0.166)	0.046
	Total n=34 (s=15)	1.020 (0.880-1.023)	0.077	0.196 (0.188-0.206)	0.003	0.112 (0.008-0.174)	0.047
Severe	Propositi n=6 (s=2)	0.923 (0.801-0.995)	0.075	0.192 (0.182-0.195)	0.005	0.099 (0.038-0.158)	0.053
	Total n=8 (s=4)	0.935 (0.801-1.023)	0.073	0.194 (0.182-0.203)	0.006	0.166 (0.038-0.176)	0.055

Mild HS: patients with normal hemoglobin and <200x10⁹/L reticulocytes. Typical HS: cases with episodic anemia, no transfusional needs and >200 x10⁹/L reticulocytes. Severe HS: subjects with serious chronic, hemolytic anemia, improving after splenectomy. Sp: spectrin, Ank: ankyrin, B3: band 3 protein, SD: standard deviation. Ranges in brackets. n: number of patients, s: number of splenectomized patients.

**Ankyrin contents corrected for circulating reticulocytes (8) according to the equation:*

% of protein 2.1 to subtract = 0.095 x reticulocytes (x10⁹/L) + 8.744.

°Includes 2 patients with increased spectrin and uncorrected ankyrin contents.

Since reticulocytosis may mask a real ankyrin deficiency, correction of ankyrin contents by reticulocytosis[3,8] was carried out. Statistics (non parametric tests) included a separated analysis of propositi to avoid a prevalence bias by the kindreds sizes.[3]

Clinical features of the HS cases with spectrin and ankyrin protein contents are shown in Table 1. Spectrin contents were lower in mild HS versus controls ($p<0.001$) and in severe versus typical HS ($p<0.01$). Ankyrin contents of patients were decreased versus controls ($p<0.001$). There were no significant differences in spectrin contents between unsplenectomized and splenectomized cases with the same clinical severity, nor in ankyrin contents among patients (exception below). Spectrin and ankyrin deficiencies occurred in 53 (85.5%) and 38 (61.3%) cases respectively, including 27 (90%) and 20 (66%) propositi.

Combined spectrin and ankyrin deficiency was the most prevalent defect (Table 2), increasing with worse clinical course. Isolated spectrin deficiency was the second most prevalent subset, being more frequent in mild disease. However, differences in relative prevalences among HS clinical groups (both propositi) were non-significant (Fisher's test).

Correction for reticulocytosis increased the prevalence of combined spectrin and ankyrin deficiencies and all patients had some ankyrin reduction ($p<0.001$), mainly in typical and severe unsplenectomized cases. Controls yielded even 15% reduction. Mean contents for unsplenectomized patients were 77.2%, 49.6% and 37.6% for mild, typical and severe HS (74.6%, 49.5% and 37.6% for the propositi).

Table 2. Erythroid membrane protein defects and clinical course of hereditary spherocytosis.

Defect	Mild HS n=20	p=10	Typical HS n=34	p=14	Severe HS n=8	p=6	Total HS n=62	p=30
Sp↓ Ank N	8 (40%)	5 (50 %)	9 (26.4%)	2 (14.3%)	2 (25%)	1 (16.6%)	19 (30%)	8 (26.6%)
Sp↓ Ank↓	9 (45%)	4 (40%)	19 (55.9%)	10 (71.4%)	6 (75%)	5 (83.3%)	34 (55%)	19 (63.3%)
Sp N Ank N	1 (5%)	1 (10%)	2 (6%)	–	–	–	3 (4.8%)	1 (3.3%)
Sp N Ank↓	2 (10%)	–	2 (6%)	1 (7.1%)	–	–	4 (6.4%)	1 (3.3%)
Sp↑ Ank↑	–	–	2 (6%)	1 (7.1%)	–	–	2 (3.2%)	1 (3.3%)

Mild HS: patients with normal hemoglobin and <200x10⁹/L reticulocytes. Typical HS: cases with episodic anemia, no transfusional needs and >200 x10⁹/L reticulocytes. Severe HS: subjects with serious chronic, hemolytic anemia, improving after splenectomy. Sp: spectrin, Ank: ankyrin, N: normal, n: total number of HS patients, p: number of propositi. Percentages (%) of cases in the subsets in brackets. Non-dominant HS: 7 propositi, 3 with typical and 4 with severe illness; 1 patient with severe HS had isolated spectrin deficiency and 6 cases had combined spectrin and ankyrin deficiencies.

Splenectomized patients had mean contents of 64.4%, and 79.1% for typical and severe HS (53.2% and 74.3% for the propositi). Corrected ankyrin contents were lower in unsplenectomized (mean 0.090, SD 0.044) versus splenectomized (mean 0.134, SD 0.046) cases with the same HS severity (p<0.001) seeming to reflect the drop of reticulocytosis after splenectomy and may suggest overcorrection by the proposed equation.[8]

As in other studies,[2,9,10] combined spectrin and ankyrin deficiency was the most prevalent abnormality in our patients. Spectrin deficiency is usually due to a primary decrease of ankyrin[2,8] and ANK1 gene should be investigated;[3,8] although in some cases association with protein 4.2 partial deficiency have been found.[10] A higher frequency of isolated spectrin deficiency and normal or increased spectrin and ankyrin contents have been reported elsewhere.[4] Our results might be due to variations in relative prevalences and methodological limitations because SDS-PAGE in continuous systems tends to undervalue protein bands. This fact could explain our small number of HS cases with band 3 deficit and the lack of significant differences in protein contents between unsplenectomized and splenectomized patients. SDS-PAGE in discontinuous systems with gradient gels yields a more accurate evaluation of protein bands.

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Key words

Hereditary Spherocytosis, laboratory diagnosis, spectrin deficiency, ankyrin deficiency, prevalence

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Acute Parvovirus B19 infection as a cause of autoimmune hemolytic anemia

A patient with homozygous beta-delta thalassemia developed a severe cold hemagglutinin disease with reticulocytopenia. Acute parvovirus B19 infection was suspected because of the presence of anti-B19 IgM in serum and viral DNA analysis in bone marrow. Evidence of recent B19 infection should be sought in patients with autoimmune hemolytic anemia and reticulocytopenia.

Sir,

Human parvovirus B19 (B19) has been reported rarely as producing autoimmune hemolytic anemia (AIHA).[1-4] In this report, a case of severe AIHA fol-