

Haptoglobin phenotypes in systemic sclerosis

Sirs,

Haptoglobin (Hp) is an acute phase protein, binding free hemoglobin and thus preventing catalysis of reactive oxygen species (1). The Hp encoding gene is polymorphic and three major subtypes, Hp1-1, Hp2-1 and Hp2-2 are the product of two genes HP1 and HP2. Hp2-2 phenotype is associated with increased prevalence of various systemic diseases, including autoimmune disorders (2). Moreover, Hp2-2 phenotype induces shift from Th1 to Th2 response and increases fibrotic processes (1, 3, 4). Systemic sclerosis (SSc) is a connective tissue disorder characterised by interstitial and perivascular fibrosis, due to different factors, including genetic, environmental, immunological and microchimeric factors (5). It has been reported that SSc is associated with Th2-type immune response and that Th1-type enhancement correlates with the improvement in SSc skin fibrosis (6-8). We examined 28 SSc outpatients (6 males, 22 females, mean age 51 ± 6.3 years), diagnosed according to ARA criteria (9), and 27 control subjects (5 males, 23 females, mean age 49 ± 7.1 years) to verify the Hp phenotype distribution. Patients and controls gave their written consent to the study. Eighteen (64.2%) of the 28 examined patients belonged to the limited SSc (ISSc), while 10 patients (35.8%) to the diffuse SSc (dSSc). Patients with ISSc presented: calcinosis (7 pts, 38%), Raynaud's phenomenon (18 pts, 100%), esophageal dysmotility (12 pts, 67%), sclerodactyly (18 pts, 100%), telangiectasia (13 pts, 72%), interstitial fibrosis lung disease (2 pts, 11%), and positivity of anti-centromer antibodies (9 pts, 50%), anti-DNA topoisomerase 1 antibodies (3 pts, 16%) and anti-RNA polymerase antibodies (1 pt, 5.5%). Patients with dSSc manifested: sclerodactyly (10 pts, 100%), chest and/or limb skin fibrosis (7 pts, 70%), Raynaud's phenomenon (9 pts, 90%), esophageal dysmotility (7 pts, 70%), interstitial fibrosis lung disease (5 pts, 50%), calcinosis (2 pts, 20%), oliguric renal failure (2 pts, 20%), telangiectasia (7 pts, 70%), tendon friction rubs (2 pts, 20%), and positivity of anti-DNA topoisomerase 1 antibodies (3 pts, 30%), anti-centromer antibodies (1 pts, 10%) and anti-RNA polymerase antibodies (4 pts, 40%). Hp phenotypes were determined in serum samples by immunoblot. Proteins were separated by 12% SDS-PAGE, transferred onto nitrocellulose

Table 1. Frequency (no. and %) of Hp phenotypes in total SSc patients, in limited and diffuse SSc patients and controls.

Groups	Hp 1-1		Hp 2-1		Hp 2-2	
	no.	%	no.	%	no.	%
SSc pts	1	3.6	9	32.1	18	64.3
ISSc pts	1	3.6	5	17.8	12	42.9
dSSc pts	0	0	4	14.3	6	21.4
Control subjects	6	22.2	12	44.4	9	33.3

and incubated with a polyclonal rabbit anti-human Hp (Dade Behring). Bands were visualised by enhanced chemiluminescence adding a secondary peroxidase-conjugated goat antibody anti-rabbit IgG. We demonstrated that Hp 2-2 phenotype was significantly more prevalent in SSc patients ($p=0.022$, $OR=3.60$, 95% CL 1.04 to 12.90, chi-square test with Mantel-Haenszel correction) and Hp 1-1 phenotype was significantly less prevalent in the same patients ($p=0.045$, $OR=0.13$, 95% CL 0.01 to 1.25, 1-tailed Fisher's exact test) (Table 1). No significant difference was found between limited and diffuse SSc patients (Table 1). Similar findings, to our knowledge, have not been previously reported in literature. Pavón and Colleagues demonstrated that an increased proportion of systemic lupus erythematosus (SLE) patients show Hp 2-2 or Hp 2-1 phenotypes (10), affirming that Hpa2 expression in SLE may contribute to some of its clinical manifestations. Our data support the hypothesis that SSc is a Th2-driven disorder (6-8) and suggest that Hp 1-1 phenotype, less prevalent in patients respect to controls, is protective toward the disease. In fact, Hp 1-1 phenotype is biologically the most effective in suppressing immunomediated inflammatory responses associated with free hemoglobin (1, 2). We believe that our study, even if preliminary, contributes to answering the following questions:

1. Is phenotype HP2-2 really implicated in the pathogenesis of SSc fibrotic process?
2. Could an early investigation of the allele variants be predictive toward SSc development?
3. Could the investigation of Hp phenotypes profile be useful to choose the specific Th1-Th2 modulating therapy?

Further investigation is necessary.

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