SHORT REPORT

Haptoglobin polymorphism in a HIV-1 seropositive Brazilian population

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Background: Haptoglobin (Hp) is a plasma protein with antioxidant and immunomodulatory properties. Three main genotypes/phenotypes (Hp1-1, Hp2-1, Hp2-2) show distinctive efficiencies in their activities and have been related to susceptibility and outcome in different diseases, including HIV infection.

Objective: To compare Hp genotype distribution between HIV-1 seropositive patients and healthy controls.

Methods: 387 Brazilian HIV-1 seropositive patients, subclassified as A, B, and C according to the Centers for Disease Control, were compared with 142 healthy controls. The influence of the polymorphism on iron status (serum iron, ferritin, transferrin, transferrin saturation), acute phase proteins (Hp, C reactive protein, fibrinogen, albumin), the HIV-1 viral load, and CD4+ T lymphocyte counts was examined.

Results: Apart from finding lower Hp concentrations among individuals with genotype Hp2-2, no other significant difference was observed.

Conclusions: No association was found between Hp genotype and either HIV status or indices of HIV progression.

Haptoglobin (Hp) is an acute phase protein with antioxidative and immunomodulatory properties.¹ Its major function is to form a stable complex with plasma free haemoglobin during episodes of intravascular haemolysis, preventing iron loss and renal damage.^{2–3}

In humans, Hp is characterised by a molecular heterogeneity with three main genotypes/phenotypes: Hp1-1, Hp2-1, and Hp2-2.^{2–4} These different proteins have distinctive efficiencies and it has been suggested that the polymorphism may have important biological consequences in several diseases.^{1–3}

In HIV infection, residual iron circulating in the plasma could enhance haemoglobin driven oxidative stress, favouring viral replication and transmission.⁵ Some clinical studies have indicated that iron metabolism is altered in patients with this infection, and that iron stores and viral load levels are higher in patients with phenotype Hp2-2,^{6–7} who also show more severely depressed CD4+ cell counts and more advanced stages of disease.^{8–9}

It has also been demonstrated that Hp may influence the Th1/Th2 lymphocytes balance by promoting a dominant Th1 cellular response, thereby affecting HIV replication or immune response to the virus.¹⁰ Moreover, there is evidence that the cellular adhesion molecules CD11/CD18, acquired by the virus during infection, significantly enhance its infectivity and cell transmission.¹¹ As Hp has been identified as an alternative ligand for these molecules, it has been suggested that it may also play a role in HIV infection susceptibility.⁸

The Brazilian population has one of the highest world prevalences of HIV infection. To our knowledge, there are no studies investigating the influence of the Hp polymorphism on this subpopulation so far. In order to verify the association between this polymorphism, virus susceptibility, and indices of infection outcome, we compared the Hp genotype frequencies between 387 HIV positive patients and 142 healthy controls, as well as the iron status (iron, ferritin, transferrin, and transferrin saturation) and the concentrations of the positive acute phase proteins (C reactive protein, Hp, fibrinogen) and negative acute phase proteins (albumin) among the different genotypes in patients and controls. CD4+ cell counts and levels of viral load were compared in the patients.

METHODS

Peripheral blood samples were collected of 387 HIV-1 seropositive adult patients (clinically classified as A (177), B (51), and C (159) according to the Centers for Disease Control¹²) who were attending the UNICAMP University Hospital in Campinas, São Paulo State, southeastern Brazil (57% men; 77% white; age 18 to 70 years). The patients had a minimum follow up time of one year from diagnosis (varying from one to 16 years), which did not differ in the three clinical groups. They were given antiretroviral therapy as recommended by the Brazilian Health Ministry (two reverse transcriptase inhibitors (nucleoside analogues/analogues plus non-analogue) plus a protease inhibitor¹³). Concomitantly, we studied a group of 142 healthy, HIV-1/HIV-2 seronegative individuals (36% men; 78% white; age 18 to 62 years), from the same geographical region.

All subjects gave their written consent for participation in the study, which was approved by the local ethics committee.

The DNA samples were obtained from leucocytes (GFX Genomic Blood Purification kit, Amersham Biosciences, Amersham, UK). Haptoglobin genotyping was undertaken by polymerase chain reaction⁴; results were initially compared with Hp phenotyping by polyacrylamide electrophoresis.¹⁴

Table 1 Haptoglobin genotype distribution in HIV-1 infected Brazilian patients and healthy controls

	Hp1-1 (%)	Hp2-1 (%)	Hp2-2 (%)	p Value
Controls (n = 142)	25.35	42.25	32.39	0.6839
Patients (n = 387)	29.20	40.05	30.75	
A (n = 177)*	31.64	42.37	25.99	0.3395
B (n = 51)	25.49	47.06	27.45	
C (n = 159)	27.67	35.22	37.11	

*A, B, and C: clinical classification according to the Centers for Disease Control (A, asymptomatic; B, mild infections; C, severe infections).

Abbreviation: Hp, haptoglobin

Table 2 Serum concentrations of iron, ferritin, transferrin saturation, transferrin, haptoglobin, C reactive protein, fibrinogen, and albumin according to haptoglobin genotype, in patients (A (n = 177), B (n = 51), and C (n = 159)) and controls (n = 142)

Variable	Patients		Controls	p Value
Iron (µg/dl)	A	Hp1-1: 95.88 (42.78)	Hp1-1: 113.58 (37.43)	0.4301
		Hp2-1: 93.31 (36.74)		
		Hp2-2: 96.41 (38.96)		
	B	Hp1-1: 101.31 (36.36)	Hp2-1: 103.65 (32.39)	
		Hp2-1: 86.17 (39.59)		
	C	Hp2-2: 103.29 (53.01)	Hp2-2: 97.52 (31.42)	
		Hp1-1: 93.14 (44.58)		
	Hp2-1: 102.36 (4.87)			
	Hp2-2: 91.73 (42.53)			
Ferritin (ng/ml)	A	Hp1-1: 132.56 (164.07)	Hp1-1: 108.21 (102.21)	0.1928
		Hp2-1: 116.72 (118.82)		
		Hp2-2: 207.52 (325.61)		
	B	Hp1-1: 179.54 (184.25)	Hp2-1: 110.61 (155.73)	
		Hp2-1: 201.34 (332.63)		
	C	Hp2-2: 119.79 (95.50)	Hp2-2: 86.11 (95.70)	
		Hp1-1: 277.51 (275.59)		
	Hp2-1: 283.24 (496.82)			
	Hp2-2: 174.31 (207.19)			
Transferrin saturation (%)	A	Hp1-1: 28.06 (12.85)	Hp1-1: 32.06 (11.59)	0.8769
		Hp2-1: 27.49 (12.73)		
		Hp2-2: 29.19 (12.93)		
	B	Hp1-1: 28.54 (10.37)	Hp2-1: 27.26 (8.56)	
		Hp2-1: 28.13 (16.72)		
	C	Hp2-2: 30.90 (16.91)	Hp2-2: 25.35 (9.03)	
		Hp1-1: 29.74 (14.22)		
	Hp2-1: 29.83 (13.83)			
	Hp2-2: 27.51 (14.19)			
Transferrin (mg/dl)	A	Hp1-1: 246.02 (60.52)	Hp1-1: 244.28 (42.07)	0.4016
		Hp2-1: 238.13 (52.62)		
		Hp2-2: 224.22 (49.55)		
	B	Hp1-1: 237.00 (30.57)	Hp2-1: 264.98 (45.97)	
		Hp2-1: 219.58 (46.31)		
	C	Hp2-2: 264.50 (78.69)	Hp2-2: 265.37 (50.01)	
		Hp1-1: 222.91 (52.33)		
	Hp2-1: 239.21 (49.70)			
	Hp2-2: 34.44 (47.13)			
Haptoglobin (mg/dl)	A	Hp1-1: 114.55 (59.08)	Hp1-1: 111.78 (34.55)	<0.0001
		Hp2-1: 107.85 (66.56)		
		Hp2-2: 88.83 (56.53)		
	B	Hp1-1: 125.48 (49.74)	Hp2-1: 116.15 (47.90)	
		Hp2-1: 101.03 (61.55)		
	C	Hp2-2: 56.73 (31.32)	Hp2-2: 93.29 (38.79)	
		Hp1-1: 126.46 (65.48)		
	Hp2-1: 109.20 (64.90)			
	Hp2-2: 101.22 (81.30)			
C reactive protein (mg/dl)	A	Hp1-1: 0.35 (0.49)	Hp1-1: 0.12 (0.12)	0.3630
		Hp2-1: 0.47 (0.98)		
		Hp2-2: 0.36 (0.47)		
	B	Hp1-1: 0.29 (0.29)	Hp2-1: 0.12 (0.11)	
		Hp2-1: 0.67 (1.28)		
	C	Hp2-2: 0.21 (0.27)	Hp2-2: 0.13 (0.10)	
		Hp1-1: 0.46 (0.48)		
	Hp2-1: 0.52 (0.68)			
	Hp2-2: 0.49 (0.70)			
Fibrinogen (mg/dl)	A	Hp1-1: 308.75 (82.80)	Hp1-1: 277.31 (49.36)	0.8793
		Hp2-1: 301.71 (67.90)		
		Hp2-2: 314.41 (75.76)		
	B	Hp1-1: 314.85 (51.22)	Hp2-1: 289.43 (57.11)	
		Hp2-1: 325.04 (96.49)		
	C	Hp2-2: 273.57 (42.14)	Hp2-2: 298.80 (51.95)	
		Hp1-1: 315.68 (81.52)		
	Hp2-1: 309.70 (84.15)			
	Hp2-2: 315.85 (86.65)			
Albumin (g/dl)	A	Hp1-1: 4.25 (0.33)	Hp1-1: 4.38 (0.32)	0.8017
		Hp2-1: 4.29 (0.40)		
		Hp2-2: 4.32 (0.42)		
	B	Hp1-1: 4.40 (0.38)	Hp2-1: 4.45 (0.31)	
		Hp2-1: 4.42 (0.39)		
	C	Hp2-2: 4.42 (0.27)	Hp2-2: 4.39 (0.34)	
		Hp1-1: 4.25 (0.41)		
	Hp2-1: 4.25 (0.51)			
	Hp2-2: 4.27 (0.38)			
CD4 ⁺ (cells/mm ³)		Hp1-1: 453.58 (302.67)		0.5899
		Hp2-1: 464.75 (292.84)		
		Hp2-2: 424.88 (253.52)		

Table 2 Continued

Variable	Patients	Controls	p Value
HIV-1 viral load (copies/ml)	Hp1-1: 17 770 (57 400) Hp2-1: 25 359 (152 297) Hp2-2: 12 615 (41 038)		0.8499

Values are mean (SD).

CD4⁺ cells ranges: A = 12 to 1596 cells/mm³; B = 28 to 1212 cells/mm³; C = 3 to 1421 cells/mm³.

Viral load ranges: A = 0 to 197 001 copies/ml; B = 0 to 208 191 copies/ml; C = 0 to 1 976 710 copies/ml.

Fibrinogen concentration was determined in plasma (Trombolyzer Analyzer, Behnk Elektronik, Norderstedt, Germany). All the other biochemical variables were measured in serum. Iron and albumin levels were assayed in a Hitachi 917 analyser (Tokyo, Japan). Ferritin concentration was determined by electrochemiluminescence in an Elecsys 1010 analyser (Hitachi), and Hp, C reactive protein, and transferrin concentrations by immunonephelometry (Dade Behring, Marburg, Germany).

The CD4⁺ cells were counted in total blood by flow cytometry (FACS Calibur, Becton Dickinson, California, USA); HIV-1 RNA plasma levels were quantified using the branched DNA method in a Bayer analyser (Leverkusen, Germany).

Statistics

Statistical analyses used the Statistical Analysis System, 8.02 for Windows, SAS Institute Inc, Cary, North Carolina, USA. Categorical variables and validity of Hardy–Weinberg equilibrium were tested by χ^2 test. Mann–Whitney, analysis of variance (ANOVA), and Tukey tests were used to compare continuous variables between patients and controls. Age and sex differences were corrected by rank transformation. The CD4⁺ cell counts and viral load levels, among Hp genotypes, were compared by the Kruskal–Wallis test. Correlations among ferritin and transferrin saturation and CD4⁺ counts, viral load, patient age, and infection time were determined by Pearson coefficient. Significance was assumed at $p < 0.05$.

RESULTS

Table 1 summarises the Hp genotype frequencies in patients and controls. The HP1 allele frequencies were of 0.49 and 0.46 in the patients and controls, respectively. Table 2 shows the comparisons of iron status and acute phase protein concentrations among the different genotypes in patients and controls, as well as the CD4⁺ counts and viral load in the patients. We also looked for correlations among ferritin and transferrin saturation, CD4⁺ counts, viral load, patient age, and infection time, but could find any.

Apart from finding lower Hp concentrations among individuals with the Hp2-2 genotype, no other significant difference was observed, even when women, individuals with raised C reactive protein levels (>0.3 mg/dl), and individuals with a reduced Hp level (<60 mg/dl) were excluded.

Although the genotype frequencies did not differ significantly between patients and controls ($p = 0.6839$) (table 1), they were not in Hardy–Weinberg equilibrium in the patient group ($p < 0.05$). The control group showed the same tendency, but the p value (0.0725) did not reach the significance level.

DISCUSSION

Several studies have related the Hp polymorphism to the susceptibility and outcome in important diseases such as diabetes mellitus, cancer, and infections, including the HIV infection.³ It has been suggested that the Hp2-2 phenotype is predisposed to greater oxidative stress, increased iron

accumulation, and more pronounced viral replication.^{6,7} These findings are, however, controversial and seem to depend on the population analysed and the variables investigated.^{15,16}

In the present study, in which we analysed a subpopulation of 387 HIV-1 seropositive Brazilian patients, all submitted to the same therapeutic protocol, the only statistically significant difference was found in the Hp levels of Hp2-2 individuals, which were about 20% lower than 2-1 and 1-1. This result has been observed in other populations.^{2,6,17} No other significant difference could be demonstrated, either regarding to the Hp genotype distributions between patients and controls and among the clinical subgroups of patients (A, B, and C), or in relation to iron status, acute phase protein levels, CD4⁺ cell counts, viral load, age of patients, or infection time. Our results are in agreement with those found by Friis *et al*¹⁸ in HIV infected pregnant Zimbabwean women, in whom an interaction between Hp phenotype and iron storage could also not be demonstrated. However, these investigators considered the ferritin levels and the Hp2-2 phenotype to be independent positive predictors of the HIV-1 viral load, which was not observed in the subpopulation analysed here.

The Brazilian population has a high level of racial miscegenation, being composed of African descendants, Europeans (Portuguese, Spanish, Italians, Germans, Dutch, Russians, and Poles, among others), Asians (Chinese, Japanese, Koreans), and immigrants from Middle Eastern countries, besides the native Indians. The HP1 allele frequencies found here were 0.49 and 0.46 in patients and controls, respectively—compatible with a mixed population, as HP1 frequencies in white American and European populations vary from 0.36 to 0.46, and in black American and African populations, from 0.52 to 0.70.²

Each Brazilian region reflects the influence of its ethnical composition, but migration among different regions is constantly occurring, particularly to the state of São Paulo, the most economically active. This could be an explanation for the lack of Hardy–Weinberg equilibrium in the patient group, and would probably also be observed in the control group if the numbers were increased.

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Take home message

No association was found between haptoglobin genotype and either HIV status or indices of HIV progression.

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