Haptoglobin polymorphism correlated with coronary artery disease

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Abstract

Introduction: Haptoglobin polymorphism has been correlated with disease and some studies have associated the Hp^2 allele with susceptibility to or protection against certain infectious (pulmonary tuberculosis, HIV) and non-infectious (diabetes, coronary artery disease, obesity) diseases. The aim of this study was to verify possible correlations of haptoglobin genotypes and subtypes by comparing coronary artery disease (CAD) patients with blood donors.
Material and methods: Haptoglobin genotypes and subtypes were analyzed by DNA amplification with the DraI restriction enzyme, in 125 CAD patients diagnosed by coronary angiography, and 125 blood donors as matched healthy controls.
Results: The distribution of haptoglobin genotypes was similar in the groups, without significant statistical differences ($p = 0.643$). The Hp^2/Hp^2 genotype was more frequent in both the CAD group and blood donors followed by Hp^2/Hp^1 and Hp^1/Hp^1. The allele frequency of Hp^2 was higher than Hp^1 in the groups. The results showed a significant difference ($p = 0.002$) between the groups regarding haptoglobin subtypes; Hp^{2FS}/Hp^{2FS} was prevalent in both groups with the least frequent subtype being Hp^{2FF}/Hp^{2FF} for CAD patients and Hp^{1F}/Hp^{1S} among blood donors. There was a statistically significant difference ($p = 0.027$) between the frequencies of the commonest allele subtype, Hp^{2FS}, and the least common, Hp^{2FF}.
Conclusions: Unlike blood donors, the Hp^{2FF}/Hp^{2FF} haptoglobin subtype was the least frequent among CAD patients, which may implicate this subtype in the development of CAD, a disease with a high mortality rate which consequently reduces the proportion of these individuals in populations.

Key words: haptoglobin polymorphism, coronary artery disease, blood donors, correlation with disease.

Introduction

Haptoglobin (Hp) is an acute-phase protein synthesized by the liver in response to inflammatory cytokines. This protein is expressed by genetic polymorphisms as three major genotypes: Hp^1/Hp^1, Hp^2/Hp^1, Hp^2/Hp^2, which are determined by the two alleles Hp^1 and Hp^2 [1, 2].

Haptoglobin consists of two chains, α- and β-chains, which are derived from a single polypeptide after proteolytic cleavage. This polypeptide exists in two versions that are correlated with the presence of the Asp-Lys (version F) or Asn-Glu (version S) amino acids at positions 52 and 53 in the α_1- and β_2-chains of haptoglobin and amino acid positions 111 and 112 in the α_2-chain [3, 4]; these versions are responsible for haptoglobin subtypes [3].

Haptoglobin polymorphisms have been implicated in parasitic diseases, infectious diseases, obesity, diabetes, coronary artery disease (CAD) and many more diseases that involve inflammatory mechanisms [5]. According to Quaye [5] studies have associated the Hp^2 allele with susceptibility to or sometimes with protection against certain infectious and non-infectious diseases, with this allele appearing to be gaining a selective advantage in several populations.

The aim of this study, to verify possible correlations between haptoglobin genotypes and subtypes in patients with CAD, was basically motivated by the studies of Delanghe *et al.* [4, 6], Golabi *et al.* [7], Bacquer *et al.* [8] who reported the prevalence of one of the haptoglobin genotypes in cardiovascular diseases, while other authors were unable to verify this association [9, 10]. Thus, this work compared CAD patients to blood donors in an attempt to correlate haptoglobin genotypes and subtypes or their allele frequencies with susceptibility to developing CAD in the Brazilian population.

Material and methods

Two groups were formed of residents of São José do Rio Preto and the surrounding region of São Paulo State, Brazil. The first group included 125 individuals diagnosed by coronary angiography as CAD patients and the second was made up of 125 apparently healthy blood donors who were selected after clinical screening. The Institution's Ethics Committee approved this study and consent was obtained from all participants.

Genomic DNA was extracted from peripheral blood leukocytes using the GE Healthcare kit [11]. Reactions of 50 μl contained 10 μl of buffer, 1.5 mM of $MgCl_2$, 10 mM of dNTPs, 5 U/μl of ready to go Taq DNA polymerase (Promega), 50-100 ng of DNA and 0.2 μmol/l of each primer of the groups: A → B, E → D or C → F, according to the methodology described by Koch *et al.* [12, 13], as follows:
Primer A: 5'-gAggggAgCTTgCCTTTCCATTg-3' (forward),
Primer B: 5'-gAgATTTTTgAgCCCTggCTggT-3' (reverse),
Primer C: 5'-CCTgCCTCgTATTAACTgCACCAT-3' (forward),
Primer D: 5'-CCgAgTgCTCCACATAgCCATgT-3' (reverse),
Primer E: 5'-gAggCgATgCCATgCAgCCTA-3' (forward),
Primer F: 5'-CATTCAggAAgTTTATCTCCA-3' (reverse).

The sequences of the Hp^1 (AC004682) and Hp^2 (M69197), supplied by the EMBL/GenBank Data Libraries, are represented by the allele subtypes Hp^{1S} and Hp^{2FS}, respectively [14, 15].

Amplification was achieved by prior denaturation at 95°C for 2 min, followed by 35 cycles at 94°C for 1 min, 69°C (A → B) or 64°C (E → D or C → F) for 2 min, 72°C for 1 min and a final extension of 72°C for 7 min.

PCR products were digested using the DraI restriction enzyme (Invitrogen), applied to electrophoresis in 1.5% agarose gel, stained with ethidium bromide solution and identified by transillumination using ultraviolet light. The haptoglobin genotypes and subtypes were confirmed according to the size of the fragments obtained (Table I).

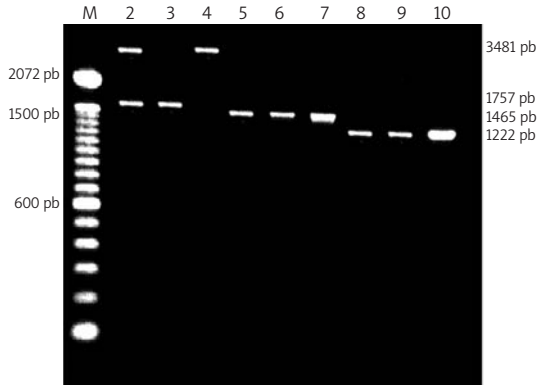
The allele frequencies were calculated for a two-allele system and differences in haptoglobin genotype and subtype frequencies between groups were compared using the chi-squared test (χ^2). A p-value ≤ 0.05 was considered significant [16].

Table I. Haptoglobin types and subtypes according to the fragments obtained by DNA amplification with DraI restriction enzyme and different primer groups

Primers Restriction	Size of fragment [pb]					Haptoglobin types (in bold type) and subtypes
A → B	3481					**Hp^2**
	1757					**Hp^1**
A → B + DraI		–	930	788	39	Hp^{1F}
A → B + DraI		1727	–	–	39	Hp^{1S}
E → D	1465					**Hp^2**
E → D + DraI		–	788	475	193	Hp^{2SS} or Hp^{2SF}
E → D + DraI		1272	–	–	193	Hp^{2FF} or Hp^{2FS}
C → F	1222					**Hp^2**
C → F + DraI		1068	–	156	–	Hp^{2FF} or Hp^{2SF}
C → F + DraI		–	927	156	139	Hp^{2SS} or Hp^{2FS}

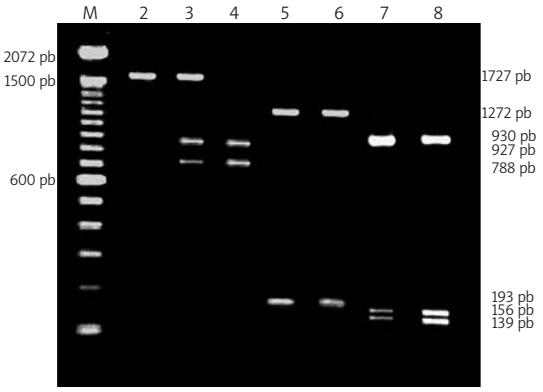
Results

Figure 1 shows products amplified with primers A and B; separated fragments may initially be translated as Hp^1/Hp^1, Hp^2/Hp^1 or Hp^2/Hp^2 genotypes and, when amplification did not occur, as the Hp^0/Hp^0 genotype. The primer pairs, E/D and C/F, identified the Hp^2/Hp^2 genotype with these amplifications requiring DraI enzyme restriction for subsequent verification of haptoglobin subtypes (Figure 2).



Lane 1 – ladder 100 pb (Invitrogen)
Lane 2 – Hp^2/Hp^1 (*primers* A → B)
Lane 3 – Hp^1/Hp^1 (*primers* A → B)
Lane 4 – Hp^2/Hp^2 (*primers* A → B)
Lane 5, 6, 7 – Hp^2/Hp^2 (*primers* E → D)
Lane 8, 9, 10 – Hp^2/Hp^2 (*primers* C → F)

Figure 1. Amplication products with primers A → B, E → D and C → F



M – DNA size marker 100 pb (Invitrogen)
Lane 2 – Hp^{1S}/Hp^{1S}
Lane 3 – Hp^{1F}/Hp^{1S}
Lane 4 – Hp^{1F}/Hp^{1F}
Lane 5, 6 – Hp^{2FF} or Hp^{2FS}
Lane 7, 8 – Hp^{2SS} or Hp^{2FS}

Figure 2. Restriction enzyme analysis with Dra I from amplifications products with *primers* A → B (lanes 2, 3 and 4), primers E → D (lanes 5 and 6) and *primers* C → F (lanes 7 and 8). Lanes 5 and 6, 7 and 8 have the same material allowing the correct characterization of subtypes

The distribution of haptoglobin genotypes in CAD patients were as follows: 31 (24.8%) homozygous Hp^1/Hp^1, 44 (35.2%) heterozygous Hp^2/Hp^1 and 48 (38.4%) homozygous Hp^2/Hp^2. Among the blood donors the distribution was 35 (28.0%) homozygous Hp^1/Hp^1, 37 (29.6%) heterozygous Hp^2/Hp^1, and 50 (40.0%) homozygous Hp^2/Hp^2. Inexpressive frequencies were found for Hp^0/Hp^0 in both groups (Table II). A comparison between CAD patients and blood donors, excluding Hp^0/Hp^0, did not identify any statistically significant differences ($p = 0.643$). The allele frequencies were similar between groups, with the frequency of the Hp^2 allele being higher than the Hp^1 allele in both groups.

The different haptoglobin subtypes were identified by amplifying the products using DraI restriction enzyme. Table III lists the subtypes with expressive percentages in the groups analyzed. Some subtypes presented with low percentages and were excluded from our results.

Hp^{2FS}/Hp^{2FS} (35.8% in CAD, 27.3% in blood donors) proved to be the most prevalent in both groups, followed by Hp^{2FS}/Hp^{1F} (22.0%) in CAD patients and Hp^{2FF}/Hp^{2FF} (15.5%) in blood donors. Hp^{2FF}/Hp^{2FF} had the lowest expression in CAD patients (1.8%). On comparing the results between the two groups, there was a statistically significant difference ($p = 0.002$) for the haptoglobin subtypes. The allele subtype frequencies for the groups showed a higher frequency for Hp^{2FS}. A comparison of the allele subtypes between the groups did not show any statistically significant difference ($p = 1.000$); however, when the Hp^{2FS} and Hp^{2FF} subtypes were considered alone, a statistically significant difference ($p = 0.027$) was observed.

Discussion

Differential susceptibility to CAD cannot be explained entirely by conventional cardiac risk factors. There is a growing awareness of the existence of polymorphic genetic loci that may act to modulate susceptibility for CAD. As oxidative stress has been strongly implicated in the atherosclerotic process, attractive candidate susceptibility genes for CAD include genes with polymorphisms which promote or protect against oxidative stress [17].

Haptoglobin is one polymorphism which has antioxidant properties; this protein binds to free haemoglobin and thereby inhibits haemoglobin-induced oxidative damage to tissues [18]. The Hp^2 protein appears to be an inferior antioxidant compared to the Hp^1 protein; thus individuals with this allele are probably more susceptible to developing inflammation and oxidative injury [19].

Our purpose in verifying possible correlations of haptoglobin genotypes and subtypes in patients with CAD was motivated basically by studies that

Table II. Type distribution and allele frequencies of haptoglobin observed in patients with coronary artery disease and in blood donors

Types and allele frequencies	CAD		Blood donors		Total	
	n	%	*n*	%	*n*	%
Hp^1/Hp^1	31	24.8	35	28.0	66	26.4
Hp^2/Hp^1	44	35.2	37	29.6	81	32.4
Hp^2/Hp^2	48	38.4	50	40.0	98	39.2
Hp^0/Hp^0	2	1.6	3	2.4	5	2.0
Total	**125**	**100**	**125**	**100**	**250**	**100**
Hp^1	0.43		0.44		0.43	
Hp^2	0.57		0.56		0.57	

χ^2 (CAD : blood donors) = 0.884; p = 0.643 (ns), (ns) – not significant

Table III. Subtype distribution and allele frequencies of haptoglobin subtypes observed in patients with coronary artery disease and in blood donors

Subtypes and allele frequencies	CAD		Blood donors		Total	
	n	%	*n*	%	*n*	%
Hp^{1S}/Hp^{1S}	15	13.8	11	10.0	26	11.9
Hp^{1F}/Hp^{1F}	7	6.4	16	14.5	23	10.5
Hp^{1F}/Hp^{1S}	9	8.3	8	7.3	17	7.7
Hp^{2FS}/Hp^{1F}	24	22.0	13	11.8	37	16.9
Hp^{2FS}/Hp^{1S}	13	11.9	15	13.6	28	12.8
Hp^{2FS}/Hp^{2FS}	39	35.8	30	27.3	69	31.5
Hp^{2FF}/Hp^{2FF}	2	1.8	17	15.5	19	8.7
Total	**109**	**100**	**110**	**100**	**219**	**100**
Hp^{1S}	0.24		0.20		0.22	
Hp^{1F}	0.21		0.24		0.23	
Hp^{2FS}	0.53		0.40		0.46	
Hp^{2FF}	0.02		0.16		0.09	

χ^2 (subtypes CAD : blood donors) = 20.621, p = 0.002(*)
χ^2 (allele frequencies CAD : blood donors) = 0.133, p = 1.000 (ns)
χ^2 (allele frequencies 2FS and 2FF CAD : blood donors) = 4.923, p = 0.027(*)
(ns) – not significant, (*) – significant

verified the prevalence of one haptoglobin genotype or one allele in cardiovascular diseases, while other authors did not verify this correlation.

Hong *et al.* [9] and Levy *et al.* [10] did not find any correlations between haptoglobin genotypes and cardiovascular disease, results similar to Chapelle *et al.* [20] and Frohlander and Johnson [21], who did not observe any association between genotype and allele frequencies with individuals who had suffered from myocardial infarction. However, Golabi *et al.* [7] reported a higher frequency of the Hp^1 allele in CAD; De Bacquer *et al.* [8] identified an elevated rate of the Hp 1-1 phenotype in patients with risk factors related to mortality due to CAD; Delanghe *et al.* [4, 6] observed a higher prevalence of Hp 2-2 phenotype in patients with peripheral arterial occlusive disease but did not observe any correlation between the phenotype distribution or allele frequencies in patients with essential arterial hypertension and Surya *et al.* [22] observed a greater prevalence of Hp 2-2 phenotype in essential hypertension.

Other authors [23-26] identified this type of correlation in patients with other diseases that may cause cardiovascular disease, reporting prevalence of the Hp 2-2 phenotype in these individuals, thereby suggesting that this phenotype makes, for example, patients with diabetes more susceptible to cardiovascular disease.

Densem *et al.* [27] compared patients who had been submitted to heart transplantation with blood donors, but the results did not demonstrate significant differences between the groups with respect to phenotype distribution. However, they

reported that among transplanted individuals the highest frequency was the Hp 2-1 phenotype, considering this phenotype to be an important prognostic tool for coronary artery diseases suggestive of transplant.

The individuals of the groups analyzed in the current study came from the same region, in other words, from a population with the same anthropological origin, thereby minimizing possible genetic variability in the distribution of polymorphisms.

Our results regarding the haptoglobin genotypes, and thus the allele frequencies, reveal that Hp^2 was commoner than Hp^1 in both the groups, so it is not possible to implicate one genotype in the presence or absence of CAD.

Moreira and Naoum [28] verified the haptoglobin phenotype distribution in a population sample from the same region and reported a higher frequency of Hp^2/Hp^1, followed by Hp^2/Hp^2 and Hp^1/Hp^1, hence different to the results found in this study.

Other works carried out in the same state in populations with similar characteristics have confirmed the prevalence of Hp^2/Hp^1 in groups of individuals considered healthy [29, 30], while another reported prevalence of Hp^2/Hp^2 [31]. Zaccariotto *et al.* [30] verified haptoglobin genotypes using a molecular methodology, while the other authors used other methodologies, which may explain this discrepancy.

The investigation of haptoglobin subtypes showed that the most prevalent in both groups was Hp^{2FS}/Hp^{2FS}. However, the second most prevalent subtype varied depending on the group: Hp^{2FS}/Hp^{1F} for patients with CAD and Hp^{2FF}/Hp^{2FF} for blood donors. It is important to mention that the second most frequent subtype in blood donors was the least common in patients with CAD.

This distribution gave a statistically significant difference, demonstrating that the ratio of the most expressed genotype (Hp^{2FS}/Hp^{2FS}) compared to the least common genotype (Hp^{2FF}/Hp^{2FF}) observed for patients with CAD is different to that observed for blood donors, which may implicate Hp^{2FF}/Hp^{2FF} in CAD.

The Hp^{2FS} allele was prevalent in both groups, with a lower frequency of Hp^{2FF}. However, the percentage of Hp^{2FF} among CAD patients was significantly lower than in blood donors, thus supporting the hypothesis that the allele is implicated in CAD.

Studies that associate subtypes with cardiovascular disease are rare. Our results agree with the results of Koch *et al.* [13], who reported a higher frequency of the Hp^{2FS} subtype and a lower frequency of the Hp^{2FF} subtype in patients who suffered from adverse effects after coronary artery interventions.

This allele, when homozygous, may favour CAD, a disease with a high mortality rate and with a consequent decrease of carriers in populations. However, it remains uncertain whether one haptoglobin genotype is responsible for protection against or susceptibility to CAD, and further studies are required with larger sample sizes to better elucidate this question.

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