Haptoglobin Genotypes and Refractory Hypertension in Type 2 Diabetes Mellitus Patients

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Abstract

Background: It has been suggested that haptoglobin polymorphism may influence the pathogenesis of microvascular and macrovascular complications in diabetic patients.

Objective: This cross sectional study was carried out to investigate the existence or not of an association between haptoglobin genotypes and prevalence of ischemic cardiovascular events (stable angina, unstable angina and acute myocardial infarction), systemic arterial hypertension, refractory hypertension, obesity and dyslipidemia in 120 type-2 diabetes mellitus patients followed up at Hospital de Clínicas da UNICAMP in Campinas, São Paulo state, southeastern Brazil.

Methods: Haptoglobin genotyping was performed by allele-specific polymerase chain reactions. The frequencies of the haptoglobin genotypes were compared with the presence/absence of cardiovascular disease, systemic arterial hypertension, refractory hypertension, obesity and dyslipidemia; systolic and diastolic blood pressure measurements; plasma levels of glucose, cholesterol (total, high density lipoprotein-HDL and low density lipoprotein-LDL) and triglycerides; and serum creatinine levels.

Results: Although no association between haptoglobin genotype and the presence of cardiovascular disease could be identified, we found a significant excess of patients with Hp2-1 genotype among those with refractory hypertension, who also had higher systolic and diastolic blood pressure, and total and LDL cholesterol levels.

Conclusion: Our results suggest that type-2 diabetes mellitus patients with the Hp2-1 genotype may have higher chances of developing refractory hypertension. Further studies in other diabetic populations are required to confirm these findings. (Arq Bras Cardiol 2011;97(4):338-345)

Keywords: Haptoglobins; hypertension; myocardial infarction; obesity; dyslipidemias; diaebtes mellitus, type 2.

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder caused by insulin resistance and beta-cell dysfunction. It is associated with high risk of developing cardiovascular disease (CVD) [1,2]. The diabetic milieu resulting from chronic hyperglycemia can generate advanced glycation end products and enhance oxidative stress, contributing directly to the progression of diabetic complications and CVD[3]. In addition, lipoproteins are oxidatively modified into oxidized low-density lipoproteins, stimulating the production of inflammatory cytokines responsible for some of the pathological changes implicated in the initiation and development of coronary heart disease, which is caused mainly by atherosclerosis[4,5].

Classic CVD risk factors and other recently discovered factors such as inflammation and oxidative stress associated with the diabetic state, however, are not sufficient to explain the occurrence of these complications, suggesting that genetic susceptibility factors influencing both glucose homeostasis and the development of atherosclerosis may be involved[6-8].

Haptoglobin (Hp) is a glycoprotein with antioxidant and immunomodulatory properties. In humans, the Hp locus is on the long arm of chromosome 16 (16q22) and is polymorphic, with two main alleles (HP1 and HP2) resulting in three distinct genotypes/phenotypes (Hp1-1, Hp2-1 and Hp2-2)[9,10]. The Hp proteins have distinct biochemical and biophysical properties, and this polymorphism has been associated with the susceptibility to and outcome of several human pathologies[11-13]. The most firmly established biological function of Hp is its strong binding to free hemoglobin (Hb)[10,14]. Clearance of the Hp-Hb complex can be mediated by the CD 163 scavenger receptor present in monocytes and macrophages. However, some changes in the glycosylation pattern of Hb that occur in diabetic patients may interfere with the binding of this complex to macrophages[15].

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It has been suggested that diabetic patients carrying the Hp2-2 genotype have increased risk of developing CVD[16-20]. However, this does not seem to be a consensual observation, and would appear to depend on the population and aspects analyzed. As far as we know, there are few studies investigating the influence of Hp polymorphism on the occurrence of ischemic cardiovascular events, systemic arterial hypertension (SAH), refractory hypertension (RH), obesity and dyslipidemia, and none of these include Brazilian patients with DM. Therefore, this is the main aim of this study.

Methods

After obtaining approval from the Local Ethics Committee, we collected peripheral blood samples from 120 T2DM patients with at least 10 years of disease followed at the Endocrinology Section of Hospital de Clínicas da UNICAMP in Campinas who had previously been investigated in another study[21] in the state of São Paulo, southeastern Brazil. All patients signed an Informed Consent Form. Pregnant patients and those aged 76 years or older were excluded from the study. The study was a cross-sectional one and was designed to investigate a possible association between Hp type and the prevalence of ischemic complications, such as stable angina, unstable angina and acute myocardial infarction with ST segment elevation and/or high markers of myocardial necrosis. Cardiovascular risk was assessed in the general group only. The presence of coronary artery disease was related to suggestive symptoms, including claudication on exertion, abnormal electrocardiogram (ECG), positive tests inducing ischemia or coronary angiography with obstructive disease. The absence of symptoms and findings described above were taken as the absence of clinically significant cardiovascular disease[22].

Stable angina was diagnosed by the presence of cardiac symptoms in a pattern that remained constant in presentation, frequency, character and duration over time and coronary disease at coronary angiography. Unstable angina was diagnosed by the presence of new cardiac symptoms and positive electrocardiogram (ECG) findings with normal biomarkers or a changing pattern of symptoms and positive ECG findings with normal biomarkers and coronary disease at coronary angiography. Acute myocardial infarction was diagnosed by ECG or diagnostic biomarkers[23]. Type 2 diabetes mellitus patients were divided into a group of 50 patients with CVD (42% males; 80% whites; aged from 42 to 75 years) and another group of 70 patients without CVD (37.14% males; 86.6% whites; aged from 48 to 75 years). All data were obtained from the patients' medical records after cardiological evaluation by a physician from the Clinical Cardiology Section of Hospital de Clínicas da UNICAMP. The demographic and clinical data of these patients and information on the medication they were taking are summarized in Table 1.

The frequencies of Hp genotypes were compared with the presence/absence of SAH, RH, obesity and dyslipidemia; systolic and diastolic blood pressure (SBP and DBP) measurements; plasma levels of glucose, cholesterol (total, HDL and LDL) and triglycerides; and serum creatinine. The whole group of T2DM patients was also compared with a control group of 142 healthy individuals (36% males; 78% whites; ages 18–62 years) who had previously been studied[24]. This group consisted of university students and employees living in the same region as the patients, who have accepted to participate in the study.

Our definition of diabetes mellitus was based on clinical features and fasting glucose of ≥ 126 mg/dL on two different occasions[25]. Glycosylated hemoglobin ($HbA1_C$) was measured using the Bio-Rad Variant II high-performance liquid chromatography system (Bio-Rad, Hercules, CA, USA) (normal range 4.5% — 6.2%). Systemic arterial hypertension was considered to be present if the SBP was >130 mm Hg and/or DPB was >90 mm Hg, and were measured in a medical visit to the Cardiology Section of Hospital de Clínicas da Unicamp. Ambulatory blood pressure monitoring (ABPM) was not performed. Refractory hypertension was considered to be present in patients being treated with three different antihypertensive medications (ideally including one diuretic drug) at effective doses if SBP >130 mm Hg and DPB >80 mm Hg[26]. Dyslipidemia was identified according to the National Cholesterol Education Program (NCEP) III guidelines as total cholesterol levels ≥200 mg/dL, or HDL <40 mg/ dL for men and <50 mg/ dL for women, or triglycerides ≥150 mg/dL[27]. Cholesterol and triglyceride levels were measured by enzymatic colorimetric assay in the Roche-Hitachi modular system (Roche Diagnostics, Mannheim, Germany). Obesity was defined as body mass index (BMI) ≥30 kg/m^2 [28]. Diabetic retinopathy (DR) and diabetic nephropathy (DN) had been previously investigated in these patients[21,29]. Serum creatinine was measured by a modified Jaffé method, and the reference values for males and females were ≤1.20 mg/dL and ≤0.90 mg/dL, respectively[30].

Genomic DNA samples were obtained from peripheral blood leukocytes (GFX Genomic Blood Purification kit, GE Healthcare, UK), and Hp genotyping was performed by allele-specific polymerase chain reaction, according to Yano et al[31].

Statistical analyses were performed with Statistical Analysis System 8.02 for Windows, SAS Institute Inc., Cary, NC, USA. Chi-square (c^2) test and Fischer exact test were used to determine whether there was an association between the categorical variables. The results of Hardy-Weinberg equilibrium were also obtained with the χ^2-test. Mann-Whitney and ANOVA tests were used to compare continuous variables. Unadjusted univariate logistic regression and multivariate logistic regression adjusted for sex and age were used to test an association between RH and some potential risk factors. P-values < 0.05 were considered statistically significant. Alpha-error of 5%, b-error of 20% and test power equal or above 80% for all statistical analyses were considered cut-off values for significance level.

Results

Table 1 shows that there is a significant difference between the diabetic patients with and without CVD in

Table 1 – Demographic characteristics (mean ± S.D.), clinical data and patterns of drug use among diabetic patients with and without CVD

Variables	T2DM patients with CVD (n = 50)	T2DM patients without CVD (n = 70)	p-values
Age, years	66.0±6.61	62.7±7.94	0.0319
Males, n (%)	21(44.7)	26(55.3)	0.5910
Whites, n (%)	32(35.6)	58(64.4)	0.3686
Duration of DM (years)	19.2±6.52	18.1±6.41	0.2568
Smoking, n (%)			
Yes	9(18.0)	4(5.7)	0.0328
No	41(82.0)	66(94.3)	
T2DM history in the family, n (%)			
Yes	4(8.0)	2(2.9)	0.2331
No	46(92.0)	68(97.2)	
SAH, n (%)			
Yes	49(98.0)	59(84.3)	0.0136
No	1(2.00)	11(15.7)	
RH, n (%)			
Yes	35(70.0)	51(72.9)	0.7320
No	15(30.0)	19(27.1)	
Dyslipidemia, n (%)			
Yes	42(84.0)	43(61.4)	0.0073
No	8(16.0)	27(38.6)	
Obesity, n (%)			
Yes	18(36.0)	23(32.9)	0.7204
No	32(64.0)	47(67.1)	
DR, n (%)			
Yes	34(68.0)	41(58.6)	0.2929
No	16(32.0)	29(41.4)	
DN, n (%)			
Yes	32(64.0)	30(42.9)	0.0223
No	18(36.0)	40(57.1)	
Neuropathy, n (%)			
Yes	27(54.0)	27(38.6)	0.0940
No	23(46.0)	43(61.4)	
Antihypertensive therapy, n (%)			
Yes	50(100.0)	57(81.4)	0.0013
No	0(0.00)	13(18.6)	
Antihyperlipidemic therapy, n (%)			
Yes	31(62.0)	32(45.7)	0.0782
No	19(38.0)	38(52.3)	
Oral antidiabetic therapy, n (%)			
Yes	29(58.0)	50(71.4)	0.1262
No	21(42.0)	20(28.6)	
Aspirin, n (%)			
Yes	47(94.0)	39(55.7)	0.0001
No	3(6.00)	31(44.3)	

S.D.- standard deviation; T2DM - Type 2 diabetes mellitus ; CVD - developing cardiovascular disease; SAH - Systemic arterial hypertension; RH - refractory hypertension; DR - Diabetic retinopathy; DN - Diabetic nephropathy.

terms of age (the mean age of the latter group is lower) and smoking habit (a higher percentage in the first group), as well as the presence of SAH, dyslipidemia and DN and, as expected, the use of antihypertensive drugs and aspirin.

A comparison of the frequencies of Hp genotypes in the two groups of patients and in the complete group of patients and the control group are shown in Table 2. Both groups of patients were in Hardy-Weinberg equilibrium (p = 0.5962) and no significant association was found.

However, when we compared the frequency distribution of the Hp genotype with the presence/absence of SAH, RH, obesity and dyslipidemia and SBP, DPB and the laboratory parameters, we found a prevalence of Hp2-1 patients with RH (p = 0.0104), as well as significant differences in SBP, DBP and total and low density lipoprotein (LDL) cholesterol (p values of 0.0113, 0.004, 0.0006 and 0.0065, respectively) (Table 3).

Univariate logistic regression analysis was used to test an association between RH and age, sex, ethnic group, obesity, high levels of total and LDL- cholesterol, triglyceride and creatinine. The analysis showed that the prevalence of RH in T2DM patients with the Hp2-1 genotype differed from that in T2DM patients with the Hp2-2 genotype (95% CI, 1.576-11.246; p = 0.0041; OR 4.210). This difference remained statistically significant in stepwise multiple logistic regression analysis (95% CI, 1.542-11.021; p = 0.0047; OR 4.123). These data are shown in Table 4.

Discussion

Diabetic state contributes to the initiation and progression of vascular complications, which remain the leading cause of mortality in diabetic patients[32]. Salles et al[33] investigated mortality rates and predictors of mortality in Brazilian T2DM patients and found evidence that these patients have a more-

Table 2 – Haptoglobin genotype in a group of Brazilian diabetic patients with or without macrovascular complications and in healthy controls

	Hp1-1 (%)	Hp2-1 (%)	Hp2-2 (%)	p-values
T2DM with macrovascular complications (n=50)	28.0	46.0	26.0	0.6954
T2DM without macrovascular complications (n=70)	21.4	48.6	30.0	
Patients (n=120)	24.2	47.5	28.3	0.6740
Controls (n=142)	25.3	42.3	32.4	

T2DM - Type 2 diabetes mellitus.

Table 3 – Presence/absence of SAH, RH, obesity and dyslipidemia; SBP and DBP (mean ± S.D.); and biochemical parameters (mean ± S.D.) of the T2DM patients divided according to Hp genotype

Blood Pressures, clinical and Biochemical data	Hp1-1	Hp2-1	Hp2-2	p-values
T2DM with SAH (n=108)	24.1	49.1	26.8	0.4919
T2DM without SAH (n=12)	25.0	33.3	41.7	
T2DM with RH (n=86)	22.1	55.8	22.1	0.0104
T2DM without RH (n=34)	29.4	26.5	44.1	
T2DM with obesity (n=41)	24.4	56.1	19.5	0.2617
T2DM without obesity (n=79)	24.1	43.0	32.9	
T2DM2 with dyslipidemia (n=85)	24.7	52.9	22.3	0.0630
T2DM2 without dyslipidemia (n=35)	22.9	34.3	42.8	
SBP (mmHg)	137.7 ± 18.9	147.5 ± 18.1	137.7 ± 20.1	0.0113
DBP (mmHg)	81.9 ± 9.5	89.6 ± 16.2	81.8 ± 11.4	0.0064
Plasma glucose (mg/dl)	156.1 ± 70.9	147.5 ± 18.1	146.4 ± 81.5	0.4180
Total cholesterol (mg/dl)	166.2 ± 34.4	195.8 ± 46.8	188.3 ± 41.2	0.0006
HDL cholesterol (mg/dl)	44.7 ± 12.2	50.8 ± 16.2	50.1 ± 14.3	0.3303
LDL cholesterol (mg/dl)	88.6 ± 27.0	115.7 ± 42.9	106.4 ± 31.8	0.0065
Triglycerides	156.1 ± 77.0	190.3 ± 46.8	156.4 ± 72.8	0.7938
Serum creatinine (mg/dl)	1.16 ± 0.81	1.32 ± 1.10	1.02 ± 0.52	0.2114

T2DM - Type 2 diabetes mellitus; SAH - Systemic arterial hypertension; RH - refractory hypertension; SBP - diastolic blood pressure; DBP - Systolic blood pressure.

Table 4 – Unadjusted univariate logistic regression analysis and multivariate logistic regression analysis adjusted for sex and age of the association between RH and the independent variables in T2DM patients

Univariate regression	Odds ratio	p-values
Age (years)	1.037	0.1781
Sex (women versus men)	2.211	0.0543
Ethnic group (white versus black)	1.083	0.8906
Obesity (yes versus no)	1.646	0.2663
Total Cholesterol (mg/dL)	1.002	0.7114
LDL (mg/dL)	1.000	0.9424
Triglycerides (mg/dL)	1.003	0.1838
Serum creatinine (mg/dL)	1.416	0.2840
Vascular complications (presence versus absence)	0.869	0.7321
Hp genotype (Hp1-1 versus Hp2-2)	1.500	0.4368
Hp genotype (Hp2-1 versus Hp2-2)	4.210	0.0041
Multivariate regression	**Odds ratio**	**p-values**
Age (years)	1.040	0.1693
Sex (women versus men)	2.129	0.0839
Hp genotype (Hp1-1 versus Hp2-2)	1.601	0.3860
Hp genotype (Hp2-1 versus Hp2-2)	4.271	0.0046

Although, due to the size sample, our data support the hypothesis of an association between the Hp genotypes and cardiovascular complications only for the patients studied here, the existence of an association between the Hp2-1 genotype and RH can be extended to the general population of T2 diabetics (test power higher than 80%).

than-three-fold excess mortality compared with the general population. According to the authors, this is partially explained by increased cardiovascular mortality. They also showed that some variables, such as older age, pre-existing vascular disease, increased 24h-proteinuria and decreased HDL cholesterol, are considered independent predictors of this increased mortality[33].

In this study, we found that the presence of CVD in T2DM Brazilian patients was associated with older age, cigarette smoking habit, SAH, dyslipidemia and development of DN. No significant differences were found for gender, duration of diabetes, family history of T2DM, RH, obesity or the presence of DR.

Haptoglobin acts as an antioxidant by protecting against the action of free radicals, and its functional allelic polymorphism is correlated with differences in protection from vascular disease[11]. A number of studies have postulated that some characteristics of the diabetic state, such as high glycosylation of the Hb molecule and reduction in the proportion of monocytes expressing surface CD163, could impair the internalization of Hb-Hp complex, especially in diabetic patients with the Hp2-2 phenotype. Furthermore, the oxidation of LDL by glycosylated Hb is apparently not completely blocked by its binding to Hp[15].

It has been suggested that the Hp phenotype is an independent risk factor for CVD in patients with diabetes mellitus and that the Hp2-2 protein may provide less protection against vascular complications than the two other types of Hp[17,19]. Levy et al[17] in a matched case-control analysis of the Strong Heart Study, which investigated a group of American Indians, observed that diabetic individuals with the Hp2-2 phenotype have five times more risk of developing CVD than those who have the Hp1-1 phenotype. An intermediate risk of CVD was found to be associated with the Hp 2-1 phenotype. However, no association was observed in individuals without diabetes mellitus[17].

In addition, Suleiman et al[19] demonstrated that the Hp phenotype is an important predictor of 30-day mortality and heart failure among individuals with diabetes and acute myocardial infarction. They found that diabetic patients with the Hp1-1 phenotype had smaller infarction sizes, whereas patients with Hp2-1 and Hp2-2 phenotypes sustained larger infarctions and consequently had a higher rate of heart failure and mortality[19].

We failed to find any significant association between Hp genotype and the presence of CVD in the diabetic patients studied here. However, when we compared the frequency of genotypes with other clinical and laboratory parameters, we found a significantly higher prevalence of Hp2-1 patients among those with RH, together with higher SBP, DBP and total and LDL-cholesterol plasma levels.

The pathogenesis of human hypertension is considered complex and multifactorial and results from the combined effect of predisposing genetic and environmental risk factors[34]. Surya Prabha et al[35] suggested that patients with the Hp 2-2 phenotype had a higher risk for essential hypertension and hypertension in association with ischemic heart disease than normal subjects. Observing that there was a significant decrease in the mean levels of serum Hp in subjects with hypertension compared with those without hypertension, they suggested the possibility of intravascular hemolysis due to vascular damage in SAH patients, and that this could lead to further complications[35]. Similarly, Delanghe et al[36] observed that hypertensive patients with the Hp2-2 phenotype need more complex combinations of antihypertensive drugs to reduce their blood pressure[36]. However, Depypere et al[37] studying patients with preeclampsia, concluded that the Hp 1-1 phenotype is associated with more severe hypertension. As far as we know, only one previous study in the literature has investigated the existence of an association between RH and Hp polymorphism. In this study, Delanghe et al[36] found that hypertensive patients with the Hp 2-2 phenotype are at higher risk of developing RH than those with Hp2-1 and Hp1-1 phenotype. All these studies, however, were carried out in nondiabetic patients.

Previous studies have found an association between the Hp2-1 phenotype and CVD. Fröhlander and Johnson[38] analyzed patients with acute myocardial infarction and found higher serum cholesterol levels in those with the Hp2-1 phenotype. Hochberg et al[39] observed that diabetic patients with the Hp 2-1 phenotype have more coronary artery collaterals than diabetic patients with the Hp2-2 phenotype in the setting of coronary artery disease. Densem et al[40] investigating the association between Hp phenotype and the development of cardiac transplant vasculopathy, observed that recipients with the Hp2-1 phenotype were more likely to develop angiographic disease. The authors suggested that the intermediate functions of Hp in Hp2-1 recipients may put these patients at disadvantage because

of the intermediate effectiveness of the Hp2-1 protein, which appears to be less effective than the Hp protein in Hp1-1 and Hp2-2 homozygotes[40]. Our findings agree with this hypothesis regarding predisposition to develop RH.

Although this study lacks a control group well matched for age, our findings make a significant contribution to the understanding of the role of Hp genotype in DM as they show that the T2DM patients with the Hp2-1 genotype studied here had a high prevalence of RH and high cholesterol levels. The frequency of this genotype in the patients with RH was 55.8%, while in patients without RH it was 26.5%. In the latter group, the frequency of the Hp2-2 genotype was 44.1%, against 22.1% in the former. Further research is required to clarify the relationship between the structural and functional properties of Hp and a predisposition to develop RH and elevated cholesterol levels, as well as the role of this protein in hypertension and its complications.

Conclusions

In conclusion, although Hp genotype cannot be used as a genetic marker for predisposition to CVD in this Brazilian population, our results suggest that T2DM patients with the Hp2-1 genotype have higher susceptibility to RH.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any post-graduation program.

References

1. Adeghate E, Schattner P, Dunn E. An update on the etiology and epidemiology of diabetes mellitus. Ann N Y Acad Sci. 2006;1084:1-29.
2. Siqueira AF, Almeida-Pititio B, Ferreira SR. Cardiovascular disease in diabetes mellitus: classical and non-classical risk factors. Arq Bras Endocrinol Metab. 2007;51(2):257-67.
3. Giugliano D, Ceriello A,Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care. 2006;19:257-67.
4. Levy AP. Genetics of diabetic cardiovascular disease: identification of a major susceptibility gene. Acta Diabetol. 2003;40(Suppl 2):S330-3.
5. Shimada K, Mokuno H, Matsunaga E, Miyazak T, Sumiyoshi K, Kume A, et al. Predictive value of circulating oxidized LDL for cardiac events in type 2 diabetic patients with coronary artery disease. Diabetes Care. 2004;27(3):843-4.
6. Eckel RH, Wassef M, Chait A, Sobel B, Barrett E, King G, et al. Prevention Conference VI: Diabetes and Cardiovascular Disease: Writing Group II: pathogenesis of atherosclerosis in diabetes. Circulation. 2002;105(18):e138-43.
7. Freedman BI, Bowden DW, Sale MM, Langefeld CD, Rich SS. Genetic susceptibility contributes to renal and cardiovascular complications of type 2 diabetes mellitus. Hypertension. 2006;18(1):8-13.
8. Fumeron F, Reis AF, Velho G. Genetics of macrovascular complications in diabetes. Curr Diab Rep. 2006:6(2):62-166.
9. Bowman BH, Kurosky A. Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. Adv Hum Genet. 1982;12:189-261.
10. Langlois MR, Delanghe JR. Biological and clinical significance of polymorphism in humans. Clin Chem. 1996;42(10):1589-600.
11. Sadrzadeh SM, Bozorgmehr J. Haptoglobin phenotypes in health and disorders. Am J Clin Pathol. 2004;121(Suppl):S97-104.
12. Carter K, Worwood M. Haptoglobin: a review of the major allele frequencies worldwide and their association with diseases. Int J Lab Hematol. 2007;29(2):92-110.
13. Wobeto VPA, Zaccariotto TR, Sonati, MF. Polymorphism of human haptoglobin and its clinical importance. Genet Mol Biol. 2008;31(3):602-20.
14. Melamed-Frank M, Lache O, Enav BI, Szafranek T, Levy NS, Rickilis RM, et al. Structure–function analysis of the antioxidant properties of haptoglobin. Blood. 2001;98(13):3693-8.
15. Levy AP, Purushothaman KR, Levy NS, Purushothaman M, Strauss M, Asleh R, et al. Downregulation of the hemoglobin scavenger receptor in individuals with diabetes and the Hp2-2 genotype: implications for the response to intraplaque hemorrhage and plaque vulnerability. Circ Res. 2007;101(1):106-10.
16. Levy AP, Roguin A, Hochberg I, Herer P, Marsh S, Nakhoul FM, et al. Haptoglobin phenotype and vascular complications in patients with diabetes. N Engl J Med. 2000;343(13):969-70.
17. Levy AP, Hochberg I, Jablonski K, Resnick HE, Lee ET, Best L, et al. Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the Strong Heart Study. J Am Coll Cardiol. 2002;40(11):1984-90.
18. Roguin A, Koch W, Kastrati A, Aronson D, Schomig A, Levy AP. Haptoglobin genotype is predictive of major adverse cardiac events in the 1-year period after percutaneous transluminal coronary angioplasty in individuals with diabetes. Diabetes Care. 2003;26(9):2628-31.
19. Suleiman M, Aronson D, Asleh R, Kapeliovich MR, Roguin A, Meisel MR, et al. Haptoglobin polymorphism predicts 30-day mortality and heart failure in patients with diabetes and acute myocardial infarction. Diabetes. 2005;54(9):2802-6.
20. Costacou T, Ferrel RE, Orchard TJ. Haptoglobin genotype: a determinant of cardiovascular complication risk in type 1 diabetes. Diabetes. 2008;57(6):1702-6.
21. Wobeto, VPA, Rosim ET, Melo MB, Calliari LEP, Sonati MF. Haptoglobin polymorphism and diabetic retinopathy in Brazilian patients. Diabetes Res Clin Pract. 2007;77(3):385-8.
22. Rajagopalan N, Miller TD, Hodge DO, Frye RL, Gibbons RJ. Identifying high-risk asymptomatic diabetic patients who are candidates for screening stress single-photon emission computed tomography imaging. J Am Coll Cardiol. 2005;45(1):43-9.
23. Luepker RV, Apple FS, Christenson RH, Crow RS, Fortmann SP, Goff D, et al. Case definitions for acute coronary heart disease in epidemiology and clinical research studies: a statement from the AHA Council on Epidemiology and Prevention; AHA Statistics Committee; World Heart Federation Council on Epidemiology and Prevention; the European Society of Cardiology Working Group on Epidemiology and Prevention; Centers for Disease Control and Prevention; and the National Heart, Lung, and Blood Institute. Circulation. 2003;108(20):2543-9.

24. Zaccariotto TR, Rosim ET, Melo D, Garcia PM, Munhoz RR, Aoki FH, et al. Haptoglobin polymorphism in a HIV-1 seropositive Brazilian population. J Clin Pathol. 2006; 59(5):550-3.

25. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabets Care. 2004;27(Suppl. 1):S5-10.

26. Lecchini R, Sabha M, Coeli FB, Fávero FF, Yugar-Toledo J, Izidoro-Toledo TC, et al. T alelle of-344C/T polymorphism in aldosterone synthase gene is not associated with resistant hypertension. Hypertens Res. 2009;32(2):159-62.

27. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA. 2001;285(19):2486-97.

28. Richard JL, Sultan A, Daures JP, Vannereau D, Parer-Richard C. Diagnosis of diabetes mellitus and intermediate glucose abnormalities in obese patients based on ADA (1997) and WHO (1985) criteria. Diabet Med. 2002;19(4):292-9.

29. Wobeto VPA, Garcia PM, Zaccariotto TR, Sonati MF. Haptoglobin polymorphism and diabetic nephropathy in Brazilian diabetic patients. Ann Hum Biol. 2009;36(4):637-41.

30. Junge W, Wilke B, Halabi A, Klein G. Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffé method. Clin Chim Acta. 2004;344(1-2):137-48.

31. Yano A, Yamamoto Y, Miyaishi S, Ishizu H. Haptoglobin genotyping by allele-specific polymerase chain reaction amplification. Acta Med Okayama. 1998;52(4):173-81.

32. Moreira LB, Fucks SC, Wiehe M, Neyeloff JL, Picon RV, Moreira MB, et al. Cardiovascular risk attributable to diabetes in southern Brazil: a population-based cohort study. Diabetes Care. 2009;32(5):854-6.

33. Salles GF, Block KV, Cardoso CR. Mortality and predictors of mortality in a cohort of Brazilian type 2 diabetic patients. Diabetes Care. 2007;27(6):1299-305.

34. Dominiczak AF, Negrin DC, Clark JS, Brosnan MJ, McBride MW, Alexander MY. Genes and hypertension: from gene mapping in experimental models to vascular gene transfer strategies. Hypertension. 2000;35(1 Pt 2):164-72.

35. Surya Prabha P, Padma T, Ramaswamy M. Haptoglobin patterns in essencial hypertension and associated conditions-increased risk for Hp2-2. Hum Hered. 1987;37(6):345-8.

36. Delanghe JR, Duprez DA, De Buyzere ML, Bergez BM, Claeys LR, Leroux-Roels, GG, et al. Refractory hypertension is associated with haptoglobin 2-2 phenotype. J Cardiovasc Risk. 1995;2(2):131-6.

37. Depypere HT, Langlois MR, Delanghe JR, Temmerman M, Dhont M. Haptoglobin polymorphism in patient with preeclampsia. Clin Chem Lab Med. 2006;44(8):924-8.

38. Fröhlander N, Johnson O. Haptoglobin groups in acute myocardial infarction. Hum Hered. 1989;39(6):345-50.

39. Hochberg I, Roguin A, Nikolsky E, Chanderashekhar PV, Cohen S, Levy AP. Haptoglobin phenotype and coronary artery collaterals in diabetic patients. Atherosclerosis. 2002;161(2):441-6.

40. Densem CG, Wassel J, Cooper A, Yonan N, Brooks NH, Keevil B. Haptoglobin phenotype correlates with development of cardiac transplant vasculopathy. J Heart Lung Transplant. 2004;23(1):43-9.