Evaluation of haptoglobin genotypes in patients with metabolic syndrome: A preliminary report

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Abstract

BACKGROUND: Haptoglobin (Hp) polymorphisms have been suggested to be associated with many pathological conditions, including cardiovascular diseases, infectious diseases, and type 2 diabetes. For the first time, we aimed to investigate the possible association between Hp genotypes and metabolic syndrome (MES) in a sample of Iranian subjects.

METHODS: In this study, 291 patients with MES according to National Cholesterol Education Program-Adult Treatment Panel III criteria, and 284 healthy individuals have been studied. We determined Hp genotype by polymerase chain reaction.

RESULTS: The frequency of three genotype (Hp1-1, Hp2-1, and Hp2-2) in healthy individuals and patients were 7.74, 39.7, 52.46, and 7.9, 31.61, 60.48 percent, respectively. There was no significant difference between the groups regarding Hp genotypes. The Hp2 allele was the predominant allele in MES (76.29%) and normal subjects (72.54%).

CONCLUSION: Hp polymorphisms are not risk factor for predisposition to MES in a sample of the Iranian population. Further studies with different ethnicities are required to validate our findings.

Keywords: Haptoglobin, Phenotype, Metabolic Syndrome

Introduction

Metabolic syndrome (MES) is a collection of cardiovascular risk factors including central obesity, hypertension, hyperglycemia, and dyslipidemia. Human haptoglobin (Hp), an acute phase protein, encoded by two major co-dominant alleles, Hp1 and Hp2, results in three functionally distinct phenotypes, Hp1-1, Hp1-2 and Hp2-2. Hp is a tetramer composed of two beta (or heavy) and two alpha (or light) chains connected by disulfide bonds. Hp is biologically the most effective hemoglobin (Hb)-binding protein and its main function are to clear tissues and circulation from this strong oxidant. Hp is a potent antioxidant playing a scavenging role for the toxic free Hb, which accumulates during acute-phase inflammatory reaction. Hp also exerts a direct angiogenic, anti-inflammatory and immunomodulatory function in extravascular tissues and body fluids. In fact in response to various stimuli, Hp is able to migrate through vessel walls and is expressed in different tissues. Furthermore, Hp can be released from neutrophil granulocytes at sites of injury or inflammation and locally dampens tissue damage. Hp receptors include CD163 expressed on the monocyte-macrophage system and CD11b (CR3) found on granulocytes, natural killer cells, and in small lymphocyte sub-populations. Hp has also been shown to bind to the majority of CD4+ and CD8+ T lymphocytes, directly inhibiting their proliferation and modifying the T-helper (Tb) Th1/Th2 balance. The Hp1-1 protein is the most effective in binding free Hb and suppressing inflammatory responses, Hp2-2 is the least active, and Hp2-1 is moderately active. The major difference among alleles Hp1 and Hp2 is the presence of a duplicated ~1.7 Kb DNA segment within Hp2, but not Hp1.

As functional differences in the antioxidant, scavenging, and immune-regulatory properties of Hp arise as a function of its polymorphism, the Hp genotypes has important biological and clinical
consequences and have been reported as risk factor for several diseases such as infections, cardiovascular, diabetes mellitus, neurological disorders, preeclampsia, and malignancies. To the best of our knowledge, there is not any report regarding the association between Hp genotypes and MES. Thus, this study was aimed to evaluate the possible association between Hp genotypes and MES in a sample of the Iranian population.

Materials and Methods

This cross-sectional study was performed on 291 MES and 284 normal subjects in Zahedan, Iran, from January 2010 to February 2011. Local Ethical Committee of Zahedan University of Medical Sciences, Iran, approved the project and informed consents were obtained from all subjects (Ethics No. 89-2053). The MES was determined as the presence of three or more of five components according to the National Cholesterol Education Program Adult Treatment Panel III as described previously. Whole blood were used for genomic DNA extraction as described previously. Sera were used for biochemical analysis.

Hp genotyping was performed using polymerase chain reaction (PCR) method described by Koch et al. The primers used were A (5-GAGGGGAGCTTGCCTTTCCATTG-3) and B (5-GAGATTTTTTGAGCCCTGGCTGGT-3) for amplification of a 1757-bp specific sequence of Hp1 allele and a 3481-bp Hp2 allele-specific sequence. Hp2 allele-specific sequence was amplified using primers C (5-GGCCCTCGTATTAACTGCAATG-3) and D (5-CCGAGTGCTCCACATAGCCATGT-3).

Target sequences were amplified in a volume of 50 µl, containing 5 µl of ×10 buffer (MgCl₂+plus) (Qiagen), 250 mM each of primers, 200 µM each of dNTP, about 0.1-10 ng genomic DNA and 2U Taq DNA polymerase. PCR condition for Hp1 and Hp2 allele-specific sequence with primers of A and B was 95 °C for 5 min, followed by 30 cycles of denaturing at 95 °C for 1 min, annealing at 69 °C for 1 min, extension at 72 °C for 2 min with a final extension cycle at 72 °C for 10 min. The temperature profile for 349-bp Hp2 allele-specific sequence with primers of C and D was 95 °C for 4 min, followed by 35 cycles of denaturing at 95 °C for 1 min, annealing at 69 °C for 1 min, extension at 72 °C for 30 s and final extension cycle at 72 °C for 10 min.

The statistical analysis of the data was performed using the SPSS for Windows (version 17, SPSS Inc., Chicago, IL, USA). Demographics and biochemical parameters between the groups were analyses by independent sample t-test for continuous data and \( \chi^2 \) test for categorical data. The associations between genotypes of Hp gene and MES were estimated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. P < 0.050 was considered statistically significant.

Results

This study consisted of 291 subjects with MES (87 males and 197 females; age 43.91 ± 14.71) and 284 normal subjects (127 males and 156 females; age 33.69 ± 13.25). The demographic and clinical characteristics of the groups are presented in table 1.

| Parameters                | MES n = 291 | Normal n = 284 | P     |
|---------------------------|-------------|----------------|-------|
| Sex (male/female)         | 87/197      | 127/156        |       |
| Age (year)                | 43.91 ± 14.71 | 33.69 ± 13.25  |       |
| FBG (mg/dl)               | 109.30 ± 44.97 | 86.14 ± 14.08  |       |
| Waist circumference (cm)  | 99.50 ± 11.61 | 82.14 ± 15.04  |       |
| Triglyceride (mg/dl)      | 183.72 ± 77.15 | 112.46 ± 48.41 | < 0.001|
| Total cholesterol (mg/dl) | 210.48 ± 45.10 | 173.34 ± 39.35 |       |
| HDL-C (mg/dl)             | 41.76 ± 6.97  | 45.45 ± 7.22   |       |
| LDL-C (mg/dl)             | 124.57 ± 40.72 | 102.49 ± 33.22 |       |
| BMI (kg/m²)               | 28.84 ± 4.65  | 23.49 ± 4.68   |       |
| Blood pressure            |             |                |       |
| Systolic (mmHg)           | 126.42 ± 21.40 | 114.34 ± 14.41 | < 0.001|
| Diastolic (mmHg)          | 80.56 ± 14.35  | 73.21 ± 10.80  |       |

MES: Metabolic syndrome; FBG: Fasting blood glucose; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; BMI: Body mass index
The genotypes and allele frequencies distribution of the Hp polymorphisms were compared among MES and normal subjects (Table 2). There were no significant differences regarding Hp polymorphism among MES and normal subjects ($\chi^2 = 4.33$, $P = 0.115$). The results showed that 23.71% of MES and 27.41% of normal subjects have Hp1 allele. No significant difference was found among the groups concerning Hp alleles (OR = 1.229, 95% CI = 0.0943-1.602, $P = 0.137$).

In addition, we calculated clinical and biochemical parameters of all subjects according to their Hp genotypes (Table 3). The results showed that there were no significant differences between genotypes and clinical/biochemical parameters ($P > 0.050$).

### Discussion

This study is the first report indicates that Hp polymorphisms are not risk factor for the development of MES. Several studies have related Hp polymorphism to susceptibility and outcome in important diseases, such as cardiovascular, hematologic and neurologic disorders, infectious diseases, malignant neoplasms and diabetes mellitus.

It is thought that genetic and environmental factors are involved in susceptibility to MES. Several candidate genes polymorphism, including FTO, paraoxonase, tumor necrosis factor-alpha, cell death-inducing DNA fragmentation factor alpha-like effector, CD36 and angiotensin-1-converting enzyme have been shown to be involved in MES.

Human plasma Hp, which is determined by two alleles Hp1 and Hp2, is classified into three common phenotypes. Hp1 is a molecule of homodimer or $(\alpha\beta)$$_2$, whereas Hp2-1 is comprised of multiple forms including homodimer, trimer, tetramer and other linear polymers. Hp2-2, on the other hand, consists of the trimer, tetramer, and other cyclic polymers. Hp2-2 phenotype is a risk factor for type 2 diabetes. Among subjects with diabetes, Hp2-2 is associated with an elevated risk to develop cardiovascular disease (CVD). Diabetic patients with Hp2-2 had impaired endothelial function compared with healthy controls and diabetic patients with Hp1-1. The Hp2-2 genotype has been associated with a higher incidence of CVD during 6-year follow-up in American Indians with diabetes as well as higher incidence of coronary artery disease during 18 years follow-up of subjects with type-1 diabetes. The Hp genotype apparently plays no role in the development or worsening of
proliferative retinopathy in diabetes mellitus 2 (DM2).35

Individuals with both DM and the Hp 2-2 genotype are at increased risk of CVD. Strategy of screening DM individuals for the Hp genotype and treating those with Hp2-2 with vitamin E appears to be highly clinically effective and significantly improves the quality of high-density lipoprotein (HDL) in Hp2-2 diabetic individuals.35,36 Reverse cholesterol transport is decreased in Hp2-2 DM. This may explain in part the increased atherosclerotic burden found in Hp2-2 DM individuals.38

No effect of the different Hp subtypes was found on total serum cholesterol, triglycerides or HDL cholesterol.39 Hp polymorphism, at least in the Korean population, does not predispose to the occurrence of CVD.40

The gene frequencies of the Hp1 and Hp2 alleles differ geographically.9 In West Africa, East Africa and South America, the Hp1 allele is predominant while North America, Europe, Asia and Australia have a predominant Hp2 allele. It has been proposed that the Hp2 have derived from the Hp1 allele in India and has a selective advantage.9 We found that Hp2 allele was predominant in our population. The limitation of this study is relatively low sample sizes. The results, therefore, need to be interpreted with caution.

Conclusion

The lack of an association between MES and polymorphisms of the Hp gene indicates that Hp genotypes cannot be genetic markers of predisposition to MES in a sample of the Iranian population. Further studies with different ethnicities are required to validate our findings.

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Conflict of Interests

Authors have no conflict of interests.

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