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Short communication

Haptoglobin phenotype and diabetic nephropathy

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Abstract

Aims/hypothesis. To determine if the haptoglobin 2 allele is associated with an increased risk for the development of diabetic nephropathy.
Methods. This study included 110 consecutive normotensive subjects with Type I (insulin-dependent) diabetes mellitus and Type II (non-insulin-dependent) diabetes mellitus seen in two outpatient clinics in Israel. Diabetes duration was greater than 10 years for Type I diabetes and more than 5 years for Type II diabetic subjects. Microalbuminuria was defined as urinary protein excretion of 30 to 300 mg/24 h, and macroalbuminuria was defined as urinary protein excretion of greater than 300 mg/24 h. Serum was taken from subjects for haptoglobin typing by gel electrophoresis.
Results. Of the participating subjects 54 had Type I and 56 had Type II diabetes. None (0/18) of the subjects homozygous for the haptoglobin 1 allele (1–1) showed any sign of diabetic nephropathy, as compared with 34 % (19/55) of subjects homozygous for the haptoglobin 2 allele (2–2) and 27 % (10/37) of heterozygous subjects (2–1) ($p < 0.04$). Of the subjects 29 showed macroalbuminuria. The risk of developing macroalbuminuria was found to be greater in subjects with two haptoglobin 2 alleles (22 %) (12/55) as compared with one haptoglobin 2 allele (8 %) (3/37) or no haptoglobin 2 alleles (0 %) (0/18) ($p < 0.03$).
Conclusion/interpretation. By showing a graded risk relation to the number of haptoglobin 2 alleles in Type I and Type II diabetic subjects, these studies further support our hypothesis that the haptoglobin phenotype is a major susceptibility gene for the development of diabetic nephropathy. [Diabetologia (2001) 44: 602–604]

Keywords Diabetic nephropathy, oxidative stress, macroalbuminuria, microalbuminuria, genetics.

Late or long-term microvascular and macrovascular complications are the leading cause of morbidity and mortality in patients with diabetes mellitus [1]. Hyperglycaemia has been shown to be a necessary but not sufficient condition for the development of these complications. Genetic differences between diabetic patients might therefore play an important part in determining why some diabetic patients develop these complications while others do not [2].

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Considerable evidence has shown the importance of the generation of reactive oxygen species in the development of diabetic vascular complications [3]. Genetically endowed differences in antioxidant protection could contribute to differential susceptibility to these complications. Haptoglobin is a haemoglobin binding serum protein which plays a major role in the protection against haeme-driven oxidative stress [4]. Transgenic mice with targeted disruption of the haptoglobin gene show a considerable increase in oxidative stress and oxidative tissue damage particularly in the kidney [5]. In humans, there are two common alleles for haptoglobin (1 and 2) manifesting as three major phenotypes 1–1, 2–1 and 2–2. Functional differences in the antioxidant capacity of the three types has been shown [4].

Table 1. Incidence of albuminuria greater than 30 mg a day segregated by haptoglobin type in Type I and Type II diabetic subjects and all subjects combined. DM, diabetes mellitus

	Haptoglobin Type			*p*
	1–1	2–1	2–2	
DM				
Type I	0/13	5/22	8/21	0.038
Type II	0/5	5/15	11/34	NS
All	0/18	10/37	19/55	0.015

Table 2. Incidence of macroalbuminuria segregated by haptoglobin type in Type I and Type II diabetic subjects and all subjects combined. DM, diabetes mellitus

	Haptoglobin Type			*p*
	1–1	2–1	2–2	
DM				
Type I	0/13	1/22	4/21	NS
Type II	0/5	2/15	8/34	NS
All	0/18	3/37	12/55	0.03

We recently reported in a series of patients with Type I (insulin-dependent) diabetes mellitus that the haptoglobin 1–1 phenotype is associated with protection against several important vascular complications in diabetes [6,7]. The aim of this study was to extend these findings to Type II diabetic patients and to assess if there was a gradient of protection provided by the dosage of haptoglobin 1 alleles.

Subjects and methods

All protocols were approved by the Human Research Ethics Committee of the Rambam Medical Center. Informed consent was obtained from all subjects.

Subjects were recruited from outpatient clinics at the Rambam Medical Center in Haifa, Israel and the Holy Family Hospital in Nazareth, Israel. Of the Type I subjects included in this study 49 were included in a previously reported study. Inclusion criteria were as follows: Type I diabetes without hypertension for at least 10 years or Type II diabetes without hypertension for at least 5 years. For all subjects a timed urine collection was obtained in the clinic for the assessment of the urinary albumin excretion (UAE). Subjects with a UAE of less than 30 mg a day were classified as not having diabetic nephropathy (DN). Microalbuminuria was defined as an UAE of 30 to 300 mg a day and macroalbuminuria was defined as an UAE of greater than 300 mg a day. Seven field fundal examinations were carried out on all subjects to detect the presence of diabetic retinopathy. Subjects with no evidence of diabetic retinopathy but with evidence of microalbuminuria or macroalbuminuria were excluded from this study as we considered very likely that the albuminuria was not because of diabetic renal disease. Included in the study were 54 Type I and 56 Type II diabetic subjects who met these inclusion criteria.

Haptoglobin phenotyping was determined from 10 ul of haemoglobin enriched serum by gel electrophoresis [8]. Briefly, a mixture of 2 ul of a 10% haemoglobin solution and 10 ul of serum were electrophoresed on a 4.7% polyacrylamide gel. Haemoglobin haptoglobin complexes were visualized using 3,3,5,5' tetramethylbenzidine. The haptoglobin phenotype of the sample was determined from the gel in a masked fashion without any knowledge of the urinary albumin excretion rate.

For statistical analysis we used a Chi squared test with Fisher exact *p* values to compare the incidence of no nephropathy, microalbuminuria and macroalbuminuria between patients with the three different haptoglobin phenotypes. We also determined if the groups were different in age, sex, age of onset of diabetes mellitus, duration of diabetes mellitus or HbA_{1c}. Categorical variables were compared by Chi squared with Fisher exact *p* values and continuous variables by the Wilcoxen independent sample test. All *p* values are based on a two-tailed comparison. A *p* value of less than 0.05 was considered to be statistically significant.

Results

We enrolled 54 patients with Type I and 56 patients with Type II diabetes in this study. There was no significant difference in the age, sex, age of onset or duration of diabetes between patients with the different haptoglobin phenotypes (p = NS). Allele frequencies for haptoglobin were consistent with those previously reported for populations of this region and were in Hardy-Weinberg equilibrium [9].

In the patients with Type I diabetes we found 13 patients who showed diabetic nephropathy as defined by albuminuria of greater than 30 mg a day. None of the 13 patients with Type I diabetes and the haptoglobin 1–1 phenotype had diabetic nephropathy, whereas 5 of 22 (23%) with haptoglobin 2–1 and 8 of 21 (38%) had evidence of diabetic nephropathy. In the patients with Type II diabetes we found 16 patients who showed diabetic nephropathy. None of the 5 patients with Type II diabetes and the haptoglobin 1–1 phenotype had diabetic nephropathy, whereas 5 of 15 (33%) with haptoglobin 2–1 and 11 of 34 patients (33%) with haptoglobin 2–2 had evidence of diabetic nephropathy. The difference in the incidence of diabetic nephropathy between patients with the three haptoglobin phenotypes was statistically significant for Type I diabetic patients and for all diabetic patients combined (Table 1).

There were 15 patients with macroalbuminuria in this study. None of the 18 diabetic patients with the haptoglobin 1–1 phenotype had macroalbuminuria whereas 3 of 37 patients with the haptoglobin 2–1 and 12 of 55 of the patients with haptoglobin 2–2 had macroalbuminuria. The difference in the incidence of macroalbuminuria between patients with the three haptoglobin phenotypes was statistically significant ($p < 0.03$) (Table 2).

Discussion

We report an extension of our previous study showing that patients with haptoglobin 1–1 are considerably protected against the development of diabetic nephropathy. This protection appears to occur in Type I and Type II diabetic patients. Furthermore, a gradient or gene dosage effect is evident, in which the number of haptoglobin 2 alleles is correlated with the development of micro and macroalbuminuria.

Haptoglobin is likely to be exerting its protective effect by its role as an antioxidant. The importance of haptoglobin in protecting against oxidative stress particularly in the kidney has been shown in transgenic mice in which the haptoglobin gene has been disrupted [5]. The relative protection afforded by the different haptoglobin phenotypes can be the result of differences in the molecular shape and size between the protein products encoded by the two different haptoglobin alleles. Smaller haptoglobin complexes might be better able to penetrate into the extracellular space and to undergo glomerular sieving. Haptoglobin circulating in the plasma is a polymer made up of haptoglobin monomers with the stoichiometry dependent upon the type of haptoglobin monomer [4]. The protein product of the haptoglobin 1 allele can cross-link with only one other haptoglobin monomer whereas the haptoglobin 2 allele protein product can cross-link with two distinct haptoglobin monomers. The net result of this difference in potential interactions is that all haptoglobin found in the subject who is homozygous for the haptoglobin 1 allele is dimeric, whereas in the subject who is a heterozygote dimers are seen (made of two haptoglobin 1 monomers) as well as linear trimers, quatermers and pentamers made up of mixtures of haptoglobin 1 and 2 products. Patients who are homozygous for the haptoglobin 2 allele can only form cyclic trimers, quatermers and pentamers.

The results of this study provide a rationale for future prospective trials to investigate the ability of vigorous antioxidant therapy to ameliorate diabetic complications in the subset of haptoglobin patients whose haptoglobin phenotype suggests that they are at greater risk. ACE inhibitor therapy has been shown to have a beneficial effect on the development and progression of diabetic nephropathy that cannot be attributed entirely to its effect on arterial blood pressure [10]. Although intraglomerular haemodynamic effects can explain in large measure the renoprotective effect of this class of agents, some studies have also suggested a role for the anti-oxidant effect of these agents. In particular, ACE inhibitors result in a reduction of haeme-driven oxidation products accumulating in the kidney and at least part of their beneficial action can be mediated by their role as antioxidants. Such beneficial effects could become more evident as study groups are stratified by haptoglobin phenotype according to the risk for oxidant mediated vascular injury.

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