

# INCREASED FREQUENCY OF HAPTOGLOBIN PHENOTYPE HP 1-1 IN DIABETIC PATIENTS WITH COMPLICATIONS

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## ABSTRACT

Distribution of Hp phenotypes was analyzed in 105 diabetic patients of whom 59 cases were with complications and 46 were without complications. Age and sex matched 110 healthy subjects served as controls. A significant increase was observed in the frequency of Hp 1-1 phenotype in patients with complications compared to controls (11.9% in cases, 0.9% in controls). Studies on haptoglobin phenotypes in diabetic cases with and without complications help us to investigate a possible genetic predisposition to diabetic complications so as to subject predisposed cases to strict glycemic control to delay the onset of complications.

**KEY WORDS:** Type-2 diabetes; Haptoglobin phenotypes; Diabetic complications.

## INTRODUCTION

Haptoglobins are alpha-2 glycoproteins synthesized in the liver and were first reported by Polonovsky and Jayle (1938)(1). They are acute phase reactants and bind to free hemoglobin released from destructed red blood cells and form Hp-Hb complexes which are removed from circulation by the liver and thus it helps in recycling of iron part of hemoglobin. This prevents renal tubule damage because of free hemoglobin deposition and also loss of iron through kidneys (2). One of the important functions of haptoglobin is to prevent loss of hemoglobin by the renal excretion (3). Haptoglobin is inherited by two co-dominant autosomal alleles situated on chromosome 16 in humans, namely Hp1 and Hp2. There are three phenotypes Hp1-1, Hp2-1 and Hp2-2. Haptoglobin molecule is a tetramer comprising of four polypeptide chains, two alpha and two beta chains of which alpha chain is responsible for polymorphism because it exists in two forms, alpha-1 and alpha-2. Hp1-1 is a combination of two alpha-1 chains along with two beta chains. Hp2-1 is a combination of one alpha-1 chain and one alpha-2 chain along with two

beta chains. Hp2-2 is a combination of two alpha-2 chains and two beta chains. Hp1-1 individuals have greater hemoglobin binding capacity when compared to those individuals with Hp2-1 and Hp2-2 (4-6).

A number of diseases have shown to be associated with different phenotypes of haptoglobin. A high frequency of Hp1-1 was observed in hemolytic anemia (7). In a study on haptoglobin phenotypes in type-2 diabetic cases, Stern et al (1986)(8) reported an increase in the frequency of Hp-1 allele with an increase in the frequency of Hp1-1 and Hp2-1 phenotypes. Haptoglobin phenotypes were shown to be associated with susceptibility to a number of vascular diseases (9). They demonstrated that greater risk of developing restenosis after coronary stent implantation in haptoglobin 2-2 (Hp2-2). Paroieva and Kazakow (2000) (10) in a study of carbohydrate metabolism disorders, reported an association of homozygous Hp1-1 and Hp2-2 in patients of pulmonary tuberculosis complicated with diabetes mellitus.

Thus, a study on distribution of haptoglobin phenotypes in diabetic cases with and without complications was undertaken by us to investigate for a possible genetic predisposition to complications of diabetes. Such studies help in identifying cases that are likely to develop complications so as to delay the onset of complications like CAD, neuropathy and nephropathy by advising the patients to maintain strict glycemic control.

## MATERIALS AND METHODS

Patients visiting the endocrinology unit of Gandhi General Hospital and Princess Durru Shehvar Hospital (Hyderabad) were selected. The clinical diagnosis of diabetes mellitus, as well as classification of cases into type-1 and type-2 diabetes, was done according to the criteria laid down by the World Health Organization (11). Blood glucose levels were estimated by glucose oxidase method (glucose oxidase kit, GOD-PAP/end point, Kaizen diagnostics, Hyderabad). None of the cases

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were found to be positive for ketosis upon withdrawal of insulin and none of the type-2 cases showed ketosis. Cases were diagnosed as suffering from type-2 diabetes mellitus as per WHO criteria. A total of 105 cases were included in the study, of these 59 were found to be suffering from complications like neuropathy, nephropathy and cardiovascular diseases. The respective specialist diagnosed each category of complications. The specialist performed necessary tests for confirmation of the complications. All the patients and controls were fasting at the time of blood collection. 2ml of venous blood was collected for haptoglobin phenotyping.

Haptoglobin phenotyping was carried out according to the method described by Giblette (1969)(7). 20µl serum of the patient was mixed with 20µl of haemolysate, prepared from human red blood cells so as to form Hp-Hb complexes. 20µl of this mixture containing 2µl of bromophenol blue dye in 40% sucrose solution was loaded for electrophoresis according to the method described by Davis (1964) (12) and benzidine was used for staining.

## RESULTS

| Category                           | Total | Hp2-2<br>n% | Hp2-1<br>n% | Hp1-1<br>n% |
|------------------------------------|-------|-------------|-------------|-------------|
| Type -2 DM<br>with complications   | 59    | 33 (55.9)   | 19 (32.2)   | 7 (11.9)    |
| Type-2 DM<br>without complications | 46    | 29 (63.0)   | 15 (32.6)   | 2 (4.3)     |
| Total type-2 DM                    | 105   | 62 (59.0)   | 34 (32.4)   | 9 (8.6)     |
| Control                            | 110   | 81 (73.6)   | 28 (25.5)   | 1 (0.9)     |

**Table-1: Distribution of Haptoglobin Phenotypes in Diabetes Mellitus and Control Subjects**

Results obtained are depicted in table-1. A perusal of the table reveals a decrease of about 17.6% in the frequency of Hp2-2 with a corresponding increase in the frequencies of Hp1-1 and Hp2-1 in diabetic patients with complications. The increase in the frequency of Hp1-1 was ten folds in cases with complications compared to that in the controls. Compared to cases without complications this increase was approximately three folds in cases with complications. The increase in the Hp1-1 frequency was found to be statistically significant when compared to controls ( $p < 0.05$ ) (table 2). In the group without complications the decrease in the frequency of Hp2-2 was about 10%. The increase in the frequency of Hp1-1 in this group was not statistically significant compared to controls.

Relative risk analysis revealed a relative risk of about 14.6 of developing diabetes and its complications for individuals with Hp1-1 phenotype. Diabetic cases with Hp1-1 phenotype have three fold increased risk of developing complications.

**Table-2: Relative Risk for Genetic Predisposition to Diabetes Mellitus with Complications**

| Hp types      | Relative risk | c 2   | P-value |
|---------------|---------------|-------|---------|
| 1-1 vs others | 14.64         | 6.10* | <0.05   |
| 1-1 vs 2-2    | 17.18         | 6.77* | <0.05   |
| 1-1 vs 2-1    | 10.31         | 4.39* | <0.05   |

\*Statistically significant at 5% level.

## DISCUSSION

The significance of haptoglobin molecules can be realized from the fact that they act as acute phase reactants. Quantitative variations in the haptoglobins indicate free radical induced damage. Haptoglobins are also important because haptoglobin types with alpha-1 chains are reported to enhance degradation of elastin by augmentation of elastase activity during an inflammatory reaction; resulting in aneurysm (13). All these aspects indicate the relevance of studies on haptoglobin phenotypes in diabetic cases in general and in those with complications, in particular. In an earlier study Stern et al (1986) (8), demonstrated an association between Hp1-1 phenotype and type-2 diabetes. They reported that haptoglobin association showed a dose effect with a single dose of Hp-1 allele with approximately 50% increase and double dose of Hp-1 allele associated with 10-fold increase in type-2 diabetes mellitus prevalence. However, the studies of Stern et al were restricted to type-2 diabetic cases without taking into consideration the complications associated with diabetes.

The objective of the present study was to investigate if there is any genetic predisposition to complications of diabetes. In view of the physiological importance of haptoglobins, we thought it is relevant to study distribution of haptoglobin phenotypes in type-2 diabetic cases with complications like neuropathy, coronary artery disease (CAD) and nephropathy and compared them with that of type-2 diabetic cases without complications. Age and sex matched healthy subjects served as controls for this study. It is interesting to note that the frequency of Hp1-1 was significantly higher in type-2 cases with complications, but in cases without complications the increase in

the frequency of Hp1-1 was not statistically significant. This indicates that Hp-1 allele in double dose predisposes to the complications of diabetes. The present study provides important information on genetic predisposition to complications. Substantial proportions of type-2 diabetic patients suffer from complications that are the major causes of morbidity and mortality in these cases. Therefore, understanding the causes of complications and genetic predisposition to these complications may help in identifying predisposed cases who can then be subjected to strict glycemic control to delay the onset of complications.

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