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POLYMORPHISM OF HUMAN HAPTOGLOBIN AND ITS IMPORTANCE IN DIABETIC NEPHROPATHY

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Haptoglobin (Hp) is a plasma glycoprotein, the main biological function of which is to bind free hemoglobin (Hb) and prevent loss of iron and subsequent kidney damage following hemolysis. In human, the Hp locus is polymorphic with two co dominant alleles, (HP_1 and HP_2) that yield three distinct genotypes/phenotypes (Hp-1-1, Hp2-1, and Hp2-2). The corresponding proteins have structural and functional difference in individual to individual. These differences may influence the susceptibility and outcome in several diseases. Here we conclude that available Polyacrylamide gel electrophoresis (PAGE) data different in different individual and altered in cases of diabetic nephropathy. Hence haptoglobin can be used as marker of susceptibility in various diseases.

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Keywords: Haptoglobin, hemoglobin, genetic polymorphism

1. Introduction

Diabetes is a major worldwide health problem, and long term diabetic vascular complications are the leading cause of morbidity and mortality [1]. With over 40 million patients, India has the largest number of diabetic patients which will touch approximately 79.4 million in the year 2030. The earlier average age of onset of Type-2 diabetes as compared to west increased the number of Indian at risk of Type-2 diabetes mainly assuming the proportions of an pandemic. Hyperglycemia has been shown to be a necessary but not sufficient condition for the development of these complications. Genetic differences between diabetic patients might play an important role in determining why some diabetic patients develop these complications while others do not [2].

The pathogenesis of diabetic nephropathy is still a matter of debate, although strong evidence suggests that it results from an interaction between susceptibility gene and the diabetic milieu. Considerable evidence has shown the importance of oxidative stress in the pathogenesis of diabetic complications, especially enhancement of atherosclerosis and diabetic micro-angiopathies.

Haptoglobin (Hp) a hepatocyte derived serum α-2-Sialoglycoprotein is a positive acute phase reactant and hemoglobin protein binding protein that they play a major role in protecting against heme-driven oxidative stress [3]. Transgenic mice with targeted disruption of the Hp gene show a considerable increase in oxidative stress and oxidative tissue damage particularly in the Kidney[4]. Haptoglobin is expressed by a genetic polymorphism as three major phenotypes – Hp1-1, Hp2-1, Hp2-2[5]. It is well established that the functional properties of Hp are type dependent.

Hp1-1 is a better antioxidant and binds more strongly with free hemoglobin than Hp2-2[6,7].The increased antioxidant function of Hp1-1 is thought to confer protection from angiopathies : however , Hp2-2 is believed to be a major risk factor in several oxidative stress related disease states[8]. Also, the Hp phenotype is an apparent risk factor for the development of gestational diabetic mellitus[9].

2. Materials and methods

10 sample (blood) collected from normal subjects and 10 sample from diabetic nephropathy cases under aseptic condition. All fine chemical of analytical grade were obtained from Sigma or Mecox India. Haptoglobin phenotype distribution was determined using 5% polyacrylamide gel electrophoresis (PAGE) as previously described by Walkley[11,12]. All chemicals for the determination of Hp Phenotyping were purchased from Sigma. A 10% of hemoglobin solution in water was heparinized blood after washing the blood cells three times in phosphate-Buffer saline (PBS).

For each sample, Hp-Hb complex solution was prepared by adding 2 μl of 10% HbA to 10 μ of serum and mixing for 4 min. at room temperature. Then, 40 ml of sample buffer (50% v/v glycerol and 0.001% w/v bromophenol blue) was added to each sample prior to running in the gel. The Hp-Hb complex was resolved by PAGE at a constant voltage of 250 V for 4 hrs. After electrophoresis was completed, the Hp-Hb complexes were visualized by immersing the gel in benzidine solution with H_2O_2 for 30 min.

Benzidine solution was freshly prepared by dissolving 200 mg of benzidine in 250 ml of boiling water. Glacial acetic acid (1.5 ml) and H_2O_2 (600 ml) were added to the Benzidine solution just prior to staining.

3.Results

The three Hp-phenotype distribution (Hp-1, Hp2-1 & Hp2-2) in normal individual (control) and diabetic nephropathy were easily distinguished by a characteristic pattern of bands representing the Hp-Hb complex as shown in Fig. 1 and Fig. 2 respectively.

Fig. 1 demonstrates that the band of Hp 1-1 in normal (control) was brighter and sharp in compared to diabetic cases band i.e. Fig. 2.

The frequency of Hp 2-2 was greater darker in diabetic nephropathy as compared to those with control (normal individual).

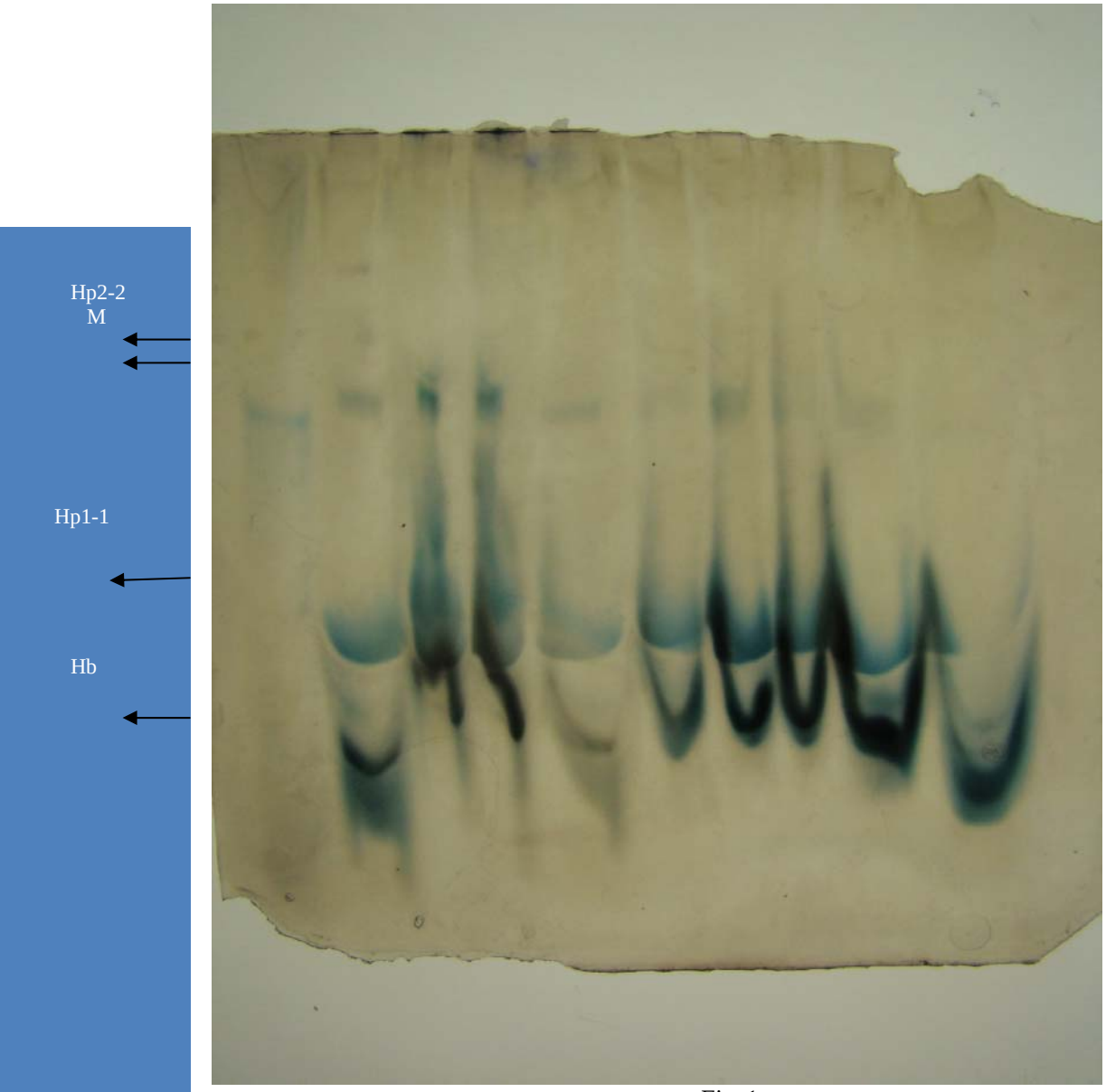

Fig. 1

M = Marker obtained from Banglore Geini (Range 14 Kda – 97 Kda)
Hb = Heamoglobin

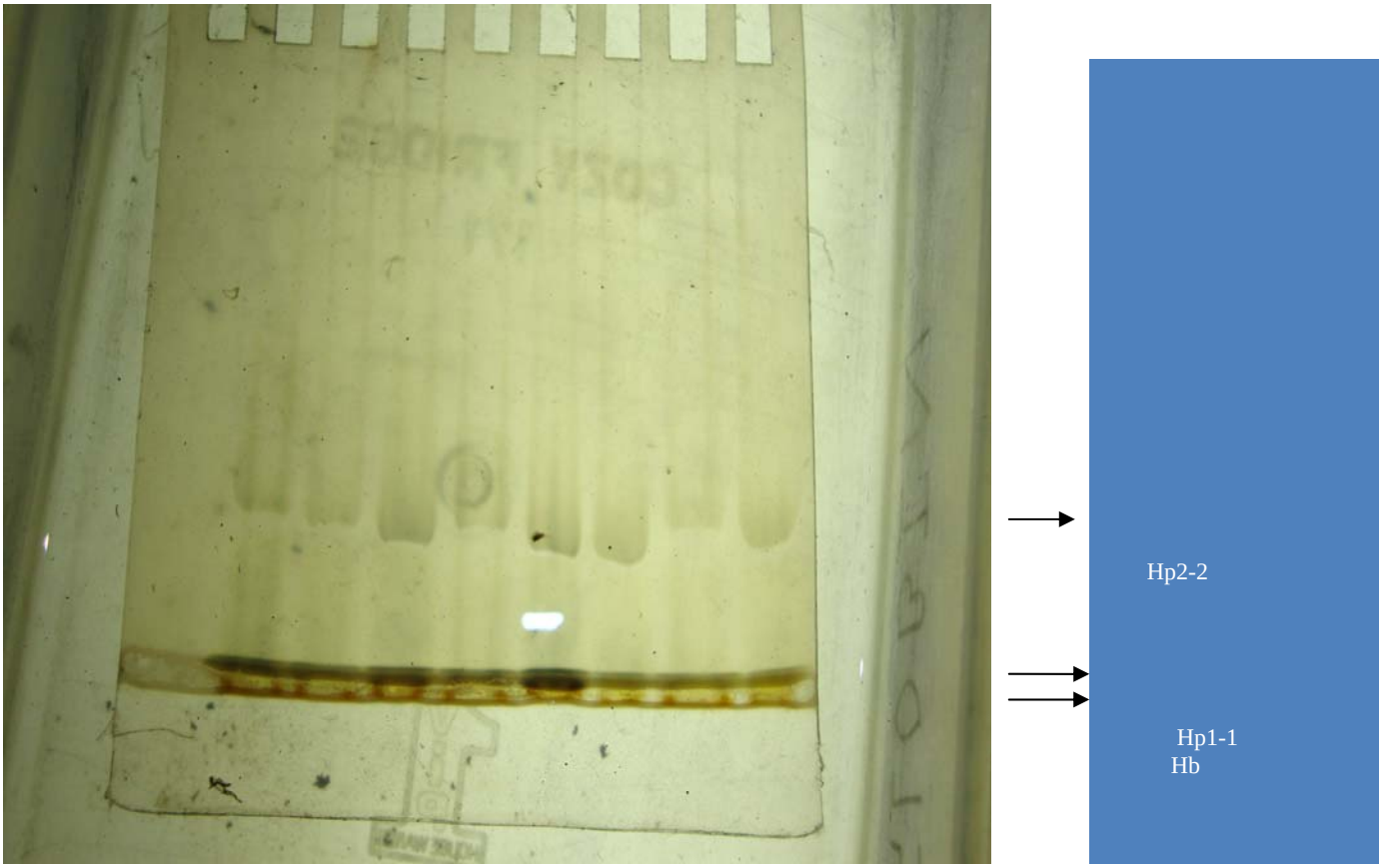

Fig. 2

4. Discussion

The chief function of Haptoglobin (Hp) is to bind to hemoglobin and thereby prevent hemoglobin induced oxidative tissue damage. This antioxidant function of Hp is mediated in part by the ability of Hp to prevent the release of iron from hemoglobin on its binding[13]. Genetically endowed differences in antioxidant protection could contribute to differential susceptibility of diabetic patients to microvascular complications including nephropathy which is the leading cause of end-stage renal disease . In human there are two common alleles for Hp(1&2), manifesting as three major phenotype Hp1-1, Hp2-1, Hp2-2[5]. This study demonstrated that the frequency of Hp1-1 was greater in normal case and bands are sharper than in those with diabetic nephropathy case whereas Hp2-2 frequency in diabetic was greater than in those of normal case and sharp and lengthy. These result are in agreement with the findings of Nakhoul et al.[14] and Levy et al.[15] who reported association of the Hp-2 allele with increase incidence of nephropathy in diabetic patients.

Screening of Type-2 diabetic patients for Hp phenotypes is essential, as Hp2-2 is considered to be a major susceptibility gene for the development of nephropathy. Measurement of serum Hp could be used as good prognostic factors for the development of nephropathy in the course of diabetic mellitus.

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