Haptoglobin Polymorphism and Diabetic Nephropathy: A Study among the Bengalee Hindus of West Bengal

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ABSTRACT

Diabetic nephropathy is a multifactorial disease that is characterized by chronic hyperglycemia, metabolic alterations and may be influenced by genetic factors. Haptoglobin (Hp) is an acute phase protein which forms 1% of the total plasma mass. The aim of the present study is to understand the association between Hp gene polymorphism and occurrence of diabetic nephropathy among the Bengalee hindus of west Bengal. A total of 87 nephropathic patients and 106 controls were recruited for the present study. Majority of the patients (79.72%) had Hp2-2 phenotype, a person having Hp2-2 phenotype are 1.89 times (95% CI 1.17-2.54) more likely to develop diabetic nephropathy than with persons with Hp2-1. Hp 2-2 phenotype is considered to be a major susceptibility gene for the development of nephropathy in type 2 diabetic patients. Haptoglobin phenotype is a significant predictor in diabetic nephropathy that demonstrates from case-control study. Hence, Hp phenotype determination can be used as a significant predictor for early prognosis of diabetic nephropathy among the Bengalee Hindus of West Bengal.

Keywords: Diabetic Nephropathy, Haptoglobin, Polymorphism, Bengalee Hindu, Case-control.

INTRODUCTION

Diabetes is a major worldwide health problem, and long-term diabetic vascular complications are the leading cause of morbidity and mortality (The DCCT Research Group, 1993). Diabetes mellitus (type-1 and type2) arises from oxidative stress resulting primarily from chronic hyperglycemia (Adinortey et al., 2011). Oxidative stress is a key component of diabetic nephropathy. A number of pathways in the kidney that generate reactive oxygen species (ROS) such as glycolysis, specific defects in the polyl pathway, uncoupling of nitric oxide synthase, xanthine oxidase, NAD(P)H oxidase, and advanced glycation have been identified as potentially major contributors to the pathogenesis of diabetic kidney disease (Forbes et al., 2008). Diabetic patients who have renal disease are characterized by structural as well as functional abnormalities such as thickening of basement membranes, mesangial expansion, hypertrophy, and glomerular epithelial cell (podocyte) loss within the glomeruli (Forbes et al., 2008). Haptoglobin (Hp), a hepatocyte-derived serum α2-sialoglycoprotein, is a positive acute-phase reactant and hemoglobin-binding protein that is essential in protecting against heme-driven oxidative stress (Bowman, 1993). Haptoglobin is expressed by a genetic polymorphism as three major phenotypes: Hp 1-1, Hp 2-1, and Hp 2-2 (Langlois and Delanghe, 1996). It is well established that the functional properties of Hp are type-dependent. Hp 1-1 is a better antioxidant and binds more strongly with free hemoglobin than Hp 2-2 (Melamed et al., 2001; Okazaki and Nagai, 1997). Genome-wide expression data through the online reference database Nephromine reveal some increase in haptoglobin mRNA expression in glomeruli and to a lesser extent in the tubuleinterstitium of patients with progressive diabetic kidney disease (Brosius and Pennathur, 2013).

According to IDF (International Diabetes Federation) diabetic atlas 2013, Indian is second position among thousand countries. In developing countries like India diabetes patients are more vulnerable to develop the micro-vascular complication like diabetic nephropathy (Viswanathan, 1999). Contemporary study (Frank et al., 2001) reported that haptoglobin gene has predictive of the risk for numerous micro-vascular and macro-vascular diabetic complications.
However, in Indian scenario the studies on Haptoglobin groups were undertaken on the issues of (Bandopadhaya et al., 1992, Bandopadhaya, 1994, Dasgupta et al., 2008, Singh et al., 2008) its polymorphic character in different population. On the other hand, selection and polymorphism of Haptoglobin groups has been studied in Bengalee population (Bandyopadhyaya, 1992, Bandyopadhyay, 1994, Bandyopadhyay, 1993, Bandyopadhyay and Ghoshal, 2002, Bandyopadhyay, 2005).

To the best of my knowledge there is no such kind of work in India as well as Bengalee population that relates the association of diabetic nephropathy with Haptoglobin groups. So, the objective of present study is to find out the association between diabetic nephropathy with Haptoglobin polymorphism in Bengalee population. The present study was aimed to understand the association between Haptoglobin gene polymorphism and diabetic nephropathy among the Bengalee hindus of west Bengal.

MATERIALS AND METHODS

The present study consists of 87 clinically diagnosed Bengalee caste Hindu male diabetic nephropathy patients (mean age 54.55±9.56 years) age ranging from 50-60 years from Fortis Hospital, Kolkata, Kidney Institute Kolkata and Nilratan Sarkar Medical College Kolkata and 105 apparently healthy individuals (having no history of diabetes in the family) from Bengalee caste Hindu males have also been collected by finger puncture in EDTA vials (55.78±7.52) years, age ranging from 50-60 years.

Polyacrylamide gel electrophoresis (PAGE) (7%) was performed to identify HP phenotypes using standard technique (Hasan et al., 2012).

Stain was prepared for identifying HP phenotypes by using TMPD (tetrathylphenylenediamine) and Tris/Hcl buffer with peroxidase activity using hydrogen peroxide following standard method (Hasan et al., 2010) with slight modification. Formed bands appeared clearly against with unstained background. The bands were identified and documented by photography (figure-2)

Allele frequencies for Haptoglobin groups were computed by Maximum Likelihood Estimation (Cavalli-Sforza and Bodmer, 1971). Statistical analysis was done by SPSS 16 software. The cut off was set as p=0.05.

RESULTS

The present study incorporates a total of 87 clinically diagnosed Bengalee Hindu male diabetic nephropathy (DN) patients and 106 healthy controls without any family history of diabetic nephropathy. All the study participants belonged to the age range of 50 – 60 years (table 1).

<table>
<thead>
<tr>
<th>Participants</th>
<th>Total (N)</th>
<th>Mean ± SD (in years)</th>
<th>Range (in years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DN</td>
<td>87</td>
<td>54.55±9.56</td>
<td>50 – 60</td>
</tr>
<tr>
<td>Controls</td>
<td>106</td>
<td>55.78±7.52</td>
<td>50 – 60</td>
</tr>
</tbody>
</table>

The above table presents the clinical profile of the patients with diabetic nephropathy. The biochemical analysis revealed that the patients are clinically determined nephropathy patients under treatment of the clinical collaborators. Haptoglobin polymorphism analysis was performed for all the individuals.

![Figure 1. Gel documentation of the Haptoglobin](image-url)
Table 2. Distribution on Hp phenotypes and allele frequencies

<table>
<thead>
<tr>
<th>Participants</th>
<th>Total</th>
<th>HP phenotype</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DN</td>
<td>87</td>
<td>0</td>
<td>22(25.28%)</td>
</tr>
<tr>
<td>Control</td>
<td>105</td>
<td>0</td>
<td>41(39.05%)</td>
</tr>
</tbody>
</table>

The above table presents the distribution of Hp phenotypes and their allele frequencies. Majority of the diabetic nephropathy patients (74.72%) have Hp2-2 phenotype, interestingly none had Hp 1-1, while majority of the controls (60.95%) had Hp 2-2 phenotype. Logistic regression analysis revealed that patients with Hp2-2 phenotypes are 1.89 times (95% CI, 1.72-2.54) likely to have nephropathy than with patients with the heterozygous state of haptoglobins.

DISCUSSION

The present study revealed Haptoglobin polymorphism as a significant predictor in diabetic nephropathy, as demonstrated from logistic regression analysis patients with Hp2-2 phenotypes are more likely to have nephropathy than with patients with Hp 2-1 phenotypes. Allele frequencies of haptoglobin in control group consistent with those previously reported for the population for this region. HP*1 frequency is higher in about 70% in Africa and America and lower frequency (15%) in India. Among Caucasian subjects HP*1 frequency decline was associated with a more rapid decline in renal function. In India, HP*1 frequency is also declining from the standard (Mourant et al., 1976) in diabetic nephropathy patients. Therefore this study suggests Haptoglobin phenotype is additional risk factor in developing diabetic nephropathy.

Several studies have established strong association between the HP phenotype and diabetic vascular complication. HP1-1 confers significant protection, HP 2-1 confers partial protection, and HP 2-2 can be regarded as a major risk factor for vascular complications in diabetes. Diabetic micro vascular complication is the leading cause of diabetic nephropathy (Martini et al., 2008).

DISCUSSION

The present findings has been consistent with previously reported study in Israel demonstrating prevalence of diabetic nephropathy in T1DM diabetic patients with HP 2-2 phenotype than HP 2-1. None of the patients have diabetic nephropathy in T1DM with HP 1-1. Similar result showing in T2DM patients (Nakhoul, 2001)

India is second position in diabetic atlas with 65.1 million diabetic people. Diabetes is large burden for India with its major complication and developing microvascular disease like diabetic nephropathy. Control of life style factors and medication are being used for prevention of diabetes (T2DM). Patients with diabetes have chance to developing diabetic nephropathy. Diabetic nephropathy creates major complication in diabetic patient and needs costly treatment. Early screening and proper therapeutic intervention might prevent diabetic patients from diabetic nephropathy. The present study envisaged that along with biochemical tests like blood sugar, creatinine, LDL etc. HP phenotype could be incorporated in screening test for diabetes and thereby diabetic nephropathy, which might have relevance in the public health.

REFERENCES


