World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

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

Abhishikta Ghosh Roy, Pranabesh Sarkar and Arup Ratan Bandyopadhyay

Human Genetics Laboratory, Department of Anthropology, University of Calcutta, Kolkata

Received: 18-02-2015 / Revised: 28-02-2015 / Accepted: 28-02-2015

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 polyol 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.

Corresponding Author Address: Abhishikta Ghosh Roy, Human Genetics Laboratory, Department of Anthropology, University of Calcutta, Kolkata, India

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 (Bandyopadhaya, 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 (tetramethylphenylenediamine) and Tris/Hcl buffer with peroxidise 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 caste 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 1. Distribution of Age of the Studied cohort

Participants	Total (N)	Mean ± SD (in years)	Range (in years)
DN	87	54.55±9.56	50 – 60
Controls	106	55.78±7.52	50 – 60

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.

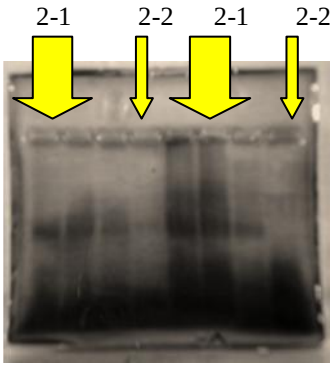

Figure 1. Gel documentation of the Haptoglobin

Table 2. Distribution on Hp phenotypes and allele frequencies

Participants	Total	HP phenotype			Allele Frequency	
	N	HP1-1	HP 2-1	HP2-2	HP*1	HP*2
DN	87	0	22(25.28%)	65(74.72%)	0.1264	0.8736
Control	105	0	41(39.05%)	64(60.95%)	0.1952	0.8048

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, 17-2.54) 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).

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

1. Adinortey MB, Gyan BA, Adjimani JP, Nyarko PE, Sarpong C, Tsikata FY, Nyarko AK, 2011. Haptoglobin polymorphism and association with complications in Ghanaian type 2 diabetic patients. *Ind J Clin Biochem*, 26(4)**:**366-372.
2. Amor AJ, Canivell S, Orila J, Ricart MJ, Hollanda AMD, Comas AB, Esmatjes E, 2014. Haptoglobin genotype and risk of diabetic nephropathy in patients with type 1 diabetes mellitus: a study on a Spanish population. *Nefrologia*, 34(2):212-215**.**
3. Asleh R, Levy AP, 2005. In vivo and in vitro studies establishing haptoglobin as a major susceptibility gene for diabetic vascular disease. *Vascular Health and Risk Management*, 1(1):19-28.
4. Bandyopadhyay AR, 1993. A parental combination analysis for ABO-HP interaction in a Bengali population. *Human Genetics*, 91(4):377-379.
5. Bandyopadhya AR, 1995. Haptoglobin and birth weight in a Bengalee population. *American Journal of Human Biology*, 7(6):711-714.
6. Bandyopadhyay AR, Roy JG, 2002. A Study on the Role of Haptoglobin in Haemolytic Disease of the Newborn. *Int J Hum Genet*, 2(3):187-195.

7. Bandyopadhyay AR, Roy JG, 2005. Interaction between haptoglobin subtypes and ABO blood groups in a Bengalee population. *Anthropol Anz*, 63 :335-340.
8. Bandopadhaya AR, Banerjee AR, Banerjee S, 1992. Haptoglobin subtypes in a Bengalee population sample. *Anthropol Anz*, 50:303-305.
9. Bandyopadhyay AR, 1994. A study on blood groups and serum proteins in Bengalee populations of Calcutta, India.*Anthropol Anz*, 52:215-9.
10. Bandopadhaya AR, 1992. Interaction between ABO and haptoglobin systems in a Bengalee population. *Hum Hered*, 42:201-203.
11. Bandyopadhyay AR, 1994. A mother-child combination analysis for ABO-HP interaction in a Bengalee population. *Hum Hered*, 44:68-71.
12. Bandyopadhyay AR, 1993. A parental combination analysis for ABO-HP interaction in a Bengali population. *Hum Genet*, 91:377-379.
13. Boger CA, Sedor JR, 2012. GWAS of Diabetic Nephropathy: Is the GENIE out of the Bottle? *PLOS Genetics*, 8(9):e1002989.
14. Brosius FC, Pennathur S, 2013. How to find a prognostic biomarker for progressive diabetic nephropathy. *Kidney International*, 83**:**996-998.
15. Carter K, Worwood M, 2007. Haptoglobin: a review of the major allele frequencies worldwide and their association with diseases. *Int. Jnl. Lab. Hem*, 29:92-110.
16. Cavalli-Sforza LL, Bodmer WF, 1971. The genetics of human populations. *W.H Freeman and Company*, San Francisco.
17. Costacou T, Ferrel RE, Ellis D, Orchard TJ, 2009. Haptoglobin Genotype and Renal Function Decline in Type 1Diabetes. *Diabetes*, 58**:** 2904-2909.
18. Daniel WW, 2010. Biostatistics. *Wiley India*, New Delhi.
19. Dasgupta S, Samtani R, Saraswathy KN, 2008. Haptoglobin polymorphism among Warli tribe of Dadra Nagar Haveli, India. *Anthropologist*, 10(4):315-316.
20. The DCCT Research Group, 1993. The effect of intensive diabetes treatment on the development and progression of long-term complications in Insulin-dependent diabetes mellitus: the Diabetes Control and Complications Trial. N Engl J Med; 329:977–86.
21. Forbes JM, Coughlan MT, Cooper ME, 2008. Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes*, 57**:**1446-1454.
22. Frank MM, Lache O, Enav BI, Szafranek T, Levy NS, Ricklis RM, Levy AP, 2001.Structure-function analysis of the antioxidant properties of haptoglobin. *Blood*, 98(3):3693-3698.
23. Galicia G, Ceppens JL, 2011. Haptoglobin Function and Regulation in Autoimmune Diseases. *Acute Phase Proteins - Regulation and Functions of Acute Phase Proteins.*
24. Gilbert RE, Cooper ME, 1999.The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury? *Kidney International*, 56**:**1627-1637**.**
25. Hamad M, Awadallah S, Nasr H, 2013.The relationship between haptoglobin polymorphism and oxidative stress in hemodialysis patients. *J Med Biochem*, 32**:**220-226.
26. Hasan M, Mohiledin A, Alzohairy M, Khan M, 2012. Human haptoglobin phenotypes on native- page using tetramethylphenylenediamine (TMPD) Staining. *Int J Biol Med Res*, 3(1):1342-1344.
27. Hasan M, Alzohairy M, Mohiledin A, 2012. Association between Haptoglobin Polymorphism and DNA damage in Type 2 diabetes. *Current Research Journal of Biological Sciences*, 4(3):284-289.
28. Maeda N, Yang F, Barnett DR, Bowman BH. Smithies O, 1984. Duplication within the haptoglobin Hp2 gene. *Nature*, 309: 131-135.
29. Martini S, Eichinger F, Nair V, Kretzler M, 2008. Defining human diabetic nephropathy on the molecular level: Integration of transcriptomic profiles with biological knowledge, *Rev Endocr Metab Disord*, 9(4): 267-274.
30. Mcevoy SM, Maeda N, 1988. Complex events in the evolution of the haptoglobin gene cluster in primates. *J. Biol.Chem*, 263: 15740-15747.
31. Mourant AE, Kopec AC, Sobczak KD, 1976. The distribution of the human blood groups and other polymorphisms. *Oxford university press*, London.
32. Nakhoul FM, Zoabi R, Kanter Y, Zoabi M, Skorecki K, Hochberg I, Leibu R, Miller B, Levy AP,2001. Haptoglobin phenotype and diabetic nephropathy. *Diabetologia*, 44:602-604.
33. Orchard JT, Sun W, Cleary PA, Genuth SM, Lachin JM, Mcgee P, Paterson AD, Raskin P, Anbinder Y, Levy AP, 2013. Haptoglobin Genotype and the Rate of Renal Function Decline in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study. *Diabetes*, 62**:**3218-3223.
34. Perdijk O, Arama C, Giusti P, Maiga B, Blomberg MT, Dolo A, Doumbo O, Perrson OJ, Bostrom S, 2013. Haptoglobin phenotype prevalence and cytokine profiles during Plasmodium falciparum infection in Dogon and Fulani ethnic groups living in Mali. *Malaria Journal*, 12:432.
35. Quaye IK, 2008. Haptoglobin, inflmation and disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102:735-742.
36. Singh HS, Saksena D, Meitei SY, Murry B, Mondal PR, Sachdeva MP, Ghosh PK, Saraswathy KN, 2012. Haptoglobin polymorphism among fourteen populations of India. *Anthropol Anz*, 69: 97-106
37. Singh HS, Saraswathy KN, 2010.Haptoglobin Polymorphism among Brahmins of Solan District Himachal Pradesh. *Anthropologist*, 12(3):211-214.
38. Smithies O, Connell GE, Dixon GH, 1962.Chromosomalrearrangements and the evolution of haptoglobin genes. *Nature*, 196: 232-236.
39. Szafranek T, Marsh S, Levy AP, 2002. Haptoglobin: A major susceptibility gene for diabetic vascular complications. *Exp Clin Cardiol*, 7(2/3):113-119.
40. Teige B, Olaisen B, Teisber P, 1992.Haptoglobin subtypes in Norway and a review of HP subtypes in various populations. *Hum Her*, 42:93-106.
41. Viswanathan V, 1999. Type 2 diabetes and diabetic nephropathy in India –magnitude of the problem. *Nephrol Dial Transplant*, 14:2805-2807.