Digest Journal of Nanomaterials and Biostructures Vol. 5, No 3, July-September 2010, p. 641-644

ANALYSIS OF THE HAPTOGLOBIN PHENOTYPE (Hp 1-1, Hp 2-1, Hp 2-2) IN CEREBRAL MALARIA CASES OF INDIAN PATIENTS

SURENDRA P. MISHRA*, ANTARA KASHYAP, ROYANA SINGH, NIDHI NARSARIA, R.K. CHATURVEDI, AMIT SINGH

Haptoglobin (Hp) is a plasma glycoprotein, the main biological function of which is to bind free hemoglobin (Hb) and prevents the loss of iron and subsequent kidney damage following hemolysis. In human, the Hp locus is polymorphic with two co-dominant alleles (Hp1 & Hp2) that yield three distinct genotype/ phenotype (Hp 1-1, Hp 2-1, Hp 2-2).The haptoglobin phenotype of Indian patients of malaria with complications like semi consciousness, coma, and uncomplicated falciparum malaria and those of uninfected randomly selected individual, were determined by electrophoresis of sera on a polyacrylamide gel (PAGE) followed by benzidine staining of the gels. Among 51 malaria (pediatric cases, 1- 8 years age group) patients, the proportion of cases with various haptoglobin phenotype, namely Hp1-1, Hp2-1, Hp2-2 were 9.5 %, 29.7% and 60.8 % respectively where as these distributions among 51 controls individual were 55.8% (Hp1-1), 18.2% (Hp2-1) and 26.0 % (Hp2-2). The available gel electrophoresis data differ in different individuals and altered in case of cerebral malaria. Hence the association of the Haptoglobin phenotype 2-2 with cerebral malaria cannot be ruled out.

(Received May 28, 2010; accepted June 19, 2010)

Keywords: Cerebral malaria, Haptoglobin, Polyacrylamide Gel Electrophoresis.

1. Introduction

Malaria is an ancient scourge of humanity. Almost half of the world's population in countries where the disease is endemic and almost every country in the world encounters in imported malaria. Malaria is the most common protozoal infections of human. The clinical picture of malaria can be variable ranging from mild to severe form leading to death. Malaria in children differs from that in adults in terms of its varied manifestations and higher mortality especially in the under five age group children.

Malarial infections results in the production of reactive oxygen species (ROS) and nitrogen species which are able to damage the surrounding tissue. Oxidative damage to DNA, RNA, protein, lipids can ultimately lead to dysfunction, disorganization and destruction of membrane, enzymes and proteins. The peroxidation of membrane lipids may cause impairment of membrane function, decrease fluidity, inactivation of membrane bound receptor and enzyme increased permeability for toxins and eventually membrane rupture.

Children with acute malaria have depressed plasma concentration of antioxidant and elevated concentration of oxidant. Raised levels of the products of lipid peroxidation in the serum are used as a marker for tissue damage and malondialdehyde (MDA) is regarded as one of the most stable products of lipid peroxidation.

During malaria infection, increased reactive oxygen species are generated that may contribute to erythrocyte damage and anemia [1], low serum copper and zinc can also contribute to the ineffective immune response of the host to antigenic challenge of the falciparum as copper and zinc are important for normal immune function.

Haptoglobin (Hp) a hepatocyte - derived serum α-2-Sialoglycoprotein is a positive acute phase reactant and hemoglobin binding protein that play a major role in protecting against heme-driven oxidative stress[2]. Haptoglobin is expressed by a genetic polymorphism as three major phenotypes – Hp1-1, Hp2-1, Hp2-2.[3] 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

*Corresponding author: drsurendram2@gmail.com.

Hp2-2. [4,5] 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.[6]

Also, the haptoglobin phenotype may be associated with susceptibility to severe *Plasmodium falciparum* malaria (cerebral malaria).[7]

2. Materials and Methods

The study was conducted in the department of Biochemistry, department of Pediatrics, Institute of medical sciences.

Fifty one blood sample were collected from strictly defined severe cerebral malaria cases and the same number from normal subjects under aseptic condition. Ethical clearance to conduct the present study was obtained from the ethical committee Institute of medical sciences, BHU. Informed consent was taken from the attendants of the patients. All fine chemicals of analytical grade were obtained from Sigma or Merck, India. Haptoglobin phenotype distribution was determined by using 5% polyacrylamide gel electrophoresis (PAGE) as previously described.[9,10] A 10% hemoglobin solution in water was prepared from heparinized blood after washing the blood cells three times in phosphate buffer saline solution(PBS). For each sample, Hp-Hb complex solution was prepared by adding 2 ml of 10% HbA to 10 ml of serum and mixing for 5 min. at room temperature. Then 40 µl of sample buffer (50% v/v glycerol and 0.001 w/v bromophenol blue) was added to each sample prior to running on 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 powder in 250 ml of boiling water. Glacial acetic acid (15 ml) and H_2O_2 (600 µl) were added to the benzidine solution just prior to staining.

3. Results

The three Hp-phenotype distributions (Hp1-1), Hp2-1 and Hp2-2) in normal individual (control) and cerebral malaria case were easily distinguished by a characteristic pattern of bands representing the Hp-Hb complex as shown in Fig. 1 and 2, respectively. Fig. 1 demonstrate that the band of Hp2-2, Hp2-1, Hp1-1 (control) group.

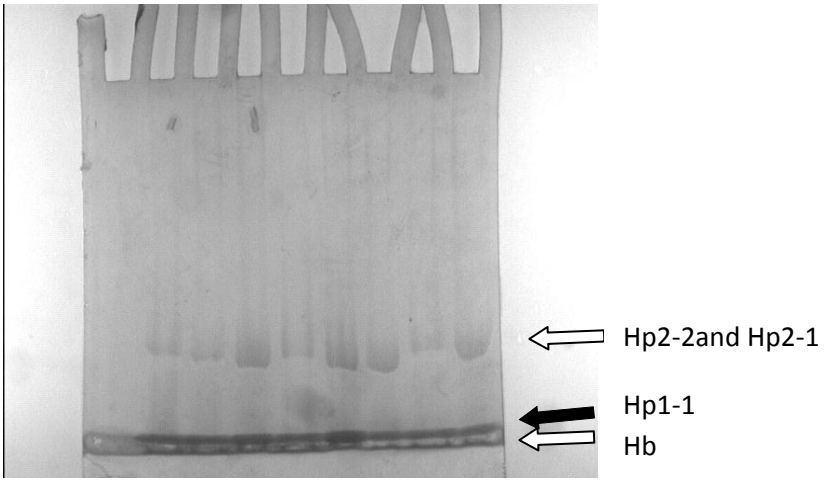
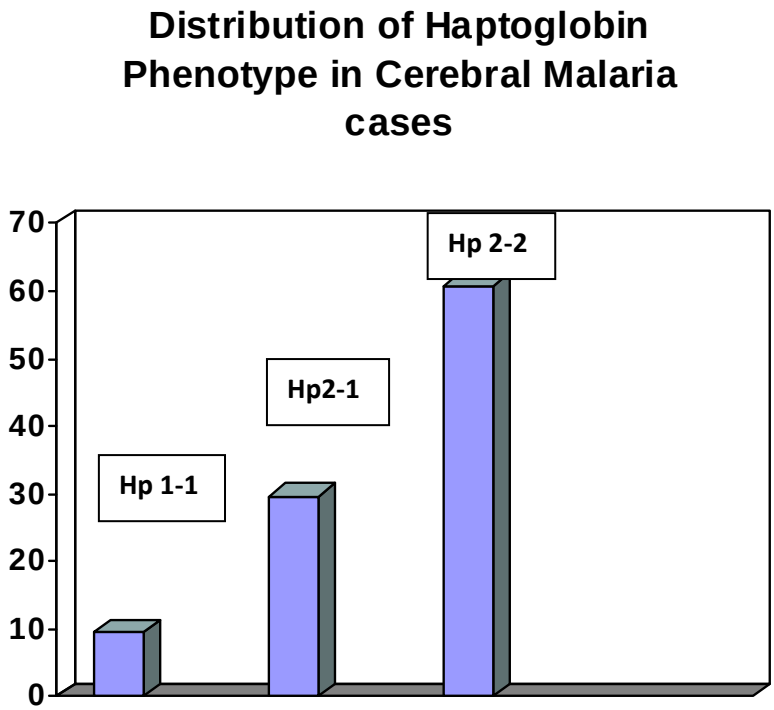


Fig 1: Hp2-2 band was lengthy and brighter, more in size as compared to cerebral malaria cases i.e. Fig. 2.

Fig. 2. The control (normal) sample in the first lane showing sharp and prominent band for Hp2-2 as compared to the samples of cases loaded in rest of the lanes.



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.[8] In human there are two co dominant alleles for Hp, namely Hp1 & Hp2, manifesting as three major phenotype 1-1, 1-2, 2-2[3]. This study demonstrated that the frequency of Hp2-2 was greater in case of cerebral malarial as compared to normal.

Thus, Hp2-2 phenotype is associated with susceptibility to severe plasmodium falciparum malaria in Varanasi region (India).

Acknowledgement

The authors are thankful to the Director, Institute of the Medical Sciences, for providing necessary facilities to carry out the work and funding of this work.

References

[1] Asleh R, Guetta J, Kalet-Litman S, Miller Lotan R, Levy AP. Haptoglobin genotypes and diabetes dependent differences in iron mediated oxidative stress in vitro and in vivo. Circ Res ; **96**: 435-41. 2005

[2] Bowman BH. Haptoglobin In Bowman BH (ed.) Haptoplasma protein, San Diego: Academic Press 1993, 159-67.

[3] Griffiths MJ, Ndungu F, Baird KL, Muller DP, Marsh K, Newton CR. Oxidative stress and erythrocyte damage in Kenyan children with severe Plasmodium falciparum malaria. Br J Haematol.; **113**: 486 (2001)

[4] Hochberg I, Roguin A, Nikolsky E, Chandrasekhar PV, Cohen S, Levy AP. Haptoglobin phenotype and coronary artery collaterals in diabetic patients. Atherosclerosis; **161**: 441 2002

[5] Quaye IK, Ekuban FA, Goka BQ, Adabayeri V, Kurtzhals JA, Gyan B, Ankrah NA, Hviid L, Akanmori BD. Haptoglobin 1-1 is associated with susceptibility to severe Plasmodium falciparum malaria. Trans R Soc Trop Med Hyg.; **94**:216 2000

[6] Langlois MR, Delanghe JR. Biologied and clinical significance of haptoglobin polymorphism in human. Clin Chem 1996; **42**: 1589-600.

[7] Melamed-Frank M, Lache O, Enav BL, Szafranek T, Levy NS, Riadis RM, Levy AP. Structure-function analysis of the antioxidant properties of Haptoglobin. Blood 2001; 98: 3693-8.

[8] Okazaki T, Nagai T. Difference in hemoglobin-bidning ability of polymers among haptoglobin phenotype. Clin Chem 1997; **43**: 2012-3.

[9] Sadrgadeh SM, Bozorgmehr J. Haptoglobin phenotypes in health and disorder. Am J Clin Pathol 2004; **121**: S97-S104 [Suppl].

[10] Wassell J, Keevil B. A new method for haptoglobin phenotyping. Am Clin Biochem 1999; 36: 609-12.