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Association of Hp 1-1 with liver disorders among Sudanese patients

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ABSTRACT

An assessment study was conducted to investigate the role of serum Haptoglobin (Hp) phenotypes in Sudanese patients with liver diseases on susceptibility to attain these diseases. Hp phenotypes were determined by separating sera supplemented with haemolysate on 4.7% polyacrylamide gel electrophoresis followed by Benzidine staining. The number of individuals with Hp1-1 was found significantly higher among patients with hepatitis B virus infection (HBV) (P<0.0005), patients with liver cirrhosis (P<0.0005) and patients with liver cancer (P<0.002) when compared to healthy control group. We suggest an association between Hp1-1 and susceptibility to HBV, liver cirrhosis and liver cancer that might be further associated with the development of different pathological complications.

Keywords: Haptoglobin- Phenotyping - Liver disorders.

INTRODUCTION

Haptoglobin (Hp) is an acute phase reactant protein, which represents 1.3% of the total plasma protein mass. HP gene is expressed in hepatocytes. Synthesis of Hp is considerably lower in fetal than in adult liver, the result of a difference in transcriptional rate (Bowman, 1993). The physiological half-life of Hp in plasma is estimated as 5.4 days (Moretti et al., 1963).

Haptoglobin major function is to bind with the free haemoglobin (Hb) released by intravascular haemolysis to form a soluble and stable Hp-Hb complex. The Hp-Hb complex is then transported to the liver to be degraded. This function of Hp is important as it prevents both iron loss and kidney damage during haemolysis. Haptoglobin has three major phenotypes: Hp1-1, Hp2-1 and Hp2-2.

In Sudan, Elagib and colleagues (Elagib et al., 1998), found that people with Hp1-1 phenotype are more susceptible to falciparum malaria and to development of severe complications, the same phenotype was also associated with other different liver diseases such as those with liver cirrhosis who showed a significant difference from controls in the distribution of Hp types, with increases in the Hp*1 allele and Hp1-1 phenotype frequencies (Zhao et al., 1993).

Increased Hp 1-1 frequency has been reported in acute myeloid leukaemia, acute lymphoid leukaemia and chronic myeloid leukaemia as well.

In the search for a molecular marker associated with the susceptibility to different types of liver diseases, few studies have been carried out on Haptoglobin phenotypes among different liver diseases in Africa and no studies of this kind are available in Sudan.

The present study was designed to investigate the distribution of Hp phenotypes among Sudanese patients with the important liver diseases: hepatitis B virus (HBV) infections, liver cirrhosis and liver cancer to detect whether an association between the
MATERIALS AND METHODS

Patients, Samples and Methods: Heparinized vacutainer tubes were used to collect venous blood samples from 267 patients with the previously mentioned liver diseases as well as from 84 healthy control donors after consent. Samples were collected basically at the Virology Department at the National Health Laboratory in Khartoum. There were 167 patients with HBV, 61 with liver cirrhosis and 45 with liver cancer. Three of the liver cancer samples were collected from the Radiation and Isotopes Centre in Khartoum Hospital and were included in the study.

The blood samples were processed at the Immunology and Biotechnology Department (Tropical Medicine Research Institute-National Center for Research), Khartoum. Blood samples were centrifuged at 200 g for 10 minutes, plasma was collected into cryotubes before freezing at -70°C for further testing.

Determination of Haptoglobin Phenotypes:
Normal erythrocyte haemolysate was prepared by washing red blood cells in phosphate-buffered saline (PBS) followed by lysis in distilled water.

Plasma samples were incubated with erythrocyte haemolysate and mixed with loading buffer (a few crystals of bromophenol blue dissolved in 40% sucrose solution) and then loaded into the gel. The above mixture was separated by discontinuous polyacrylamide gel (non-reducing) according to Davis and colleagues (Davis and Colleagues, 1968). The separation gel (Resolving gel) concentration was 4.7% polyacrylamide, and the stacking gel was 2.5%.

Gels were then stained for 10-15 minutes using benzidine stain (0.2 gm benzidine powder, 40 ml H2O, 15 ml methanol, 5 ml glacial acetic acid and 60 μl H2O2), washed in distilled water before drying and photography and haptoglobin phenotype determined according to banding pattern.

Data Analysis: The Statistical Package for Social Sciences (SPSS), Chi²-test was used to determine the association between the distribution of Hp phenotypes among different types of liver disorders and healthy control group. Charts were prepared using MS Excel.

RESULTS

In the gel, Hp1-1 phenotype appears as only one thick fast or slow migrating band closer to the free haemoglobin. Hp2-1 phenotype appears as multiple fine, slow moving bands and have the same thickness in addition to a band that corresponds to the Hp1-1 whereas Hp2-2 phenotype shows a series of multiple fine and closer to each other bands with the fastest migrating band appears fainter than its preceding band (Fig.1).

Haptoglobin phenotypes of liver diseases and healthy controls: The haptoglobin phenotypes of 276 individuals with different liver diseases, and 84 healthy controls were determined (Table 1). In all cases, the distribution of haptoglobin phenotypes among 84 healthy controls was: 20.2% (n = 17) had Hp1-1 phenotype, 48.8% (n = 41) had Hp2-1 phenotype and 31.0% (n = 26) had Hp2-2 phenotype.

Patients with Hepatitis B Virus infection (HBV): A significant difference was observed between patients with HBV and healthy controls (χ²-test, P=0.0005) in Hp1-1 phenotype distribution. The detailed distribution of haptoglobin phenotypes among 167 patients with HBV infection was: 52.7% (n = 88) had
Hp1-1 phenotype, 29.9% (n = 50) had Hp2-1 phenotype and 17.4% (n = 29) had Hp2-2 phenotype. (Table 1; Figure 2).

Fig2: Distribution of haptoglobin phenotypes among patients with different liver diseases and healthy controls.

Patients with Liver cirrhosis: The haptoglobin phenotypes distribution among 61 patients with liver cirrhosis was: 65.6% (n=40) had Hp1-1 phenotype, 23.0% (n=14) had Hp2-1 phenotype and 11.5% (n=7) had Hp2-2 phenotype. There was a significant increase in the Hp1-1 phenotype among patients with liver cirrhosis when compared with the healthy control group (p = 0.0005, χ² test).

Patients with Liver cancer: The haptoglobin phenotypes distribution among 48 patients with liver cancer was: 50.0% (n=24) had Hp1-1 phenotype, 29.2% (n=14) had Hp2-1 phenotype and 20.8% (n=10) had Hp2-2 phenotype (Figure 2). There was a significant increase in the Hp1-1 phenotype among liver cancer patients when compared with the healthy control group (p = 0.002, χ² test).

DISCUSSIONS:
As a significant increase in Hp1-1 phenotypes was found among patients with liver cancer, when they were compared to the healthy controls. Other studies on different liver cancer reported the same findings. Hp1-1 frequency has been reported to increase in patients with chronic hepatities B (Padma et al., 1988) and individuals with Hp1-1 phenotypes reported to show increased risk for chronic hepatitis C (Louagie et al., 1996).

In this study a significant increase in the frequency of Hp1-1 phenotype was observed among patients with all three liver diseases including liver cirrhosis which agrees with (Blenk et al., 1978; Zhao et al., 1993), but disagreed with (Vitalis et al., 2011).

Therefore, it is reasonable to conclude that individuals with Hp1-1 phenotypes might be more susceptible to liver cancer than individuals with other Hp phenotypes however, this needs more confirmation at the molecular and immunological levels.

REFERENCES:


