Haptoglobin

Introduction

Haptoglobin is an alpha-two glycoprotein synthesized in the liver which is capable of forming equimolar noncovalent complexes with hemoglobin. The relatively large hemoglobin–haptoglobin complex is retained by the glomerular basement membrane and metabolized by reticuloendothelial cells in the liver, bone marrow, and spleen, conserving hemoglobin iron. The haptoglobin molecule has two alpha chains, showing genetic polymorphism, and two beta chains containing the hemoglobin binding sites. Haptoglobin binds oxyhemoglobin, methemoglobin, free alpha chains, alpha–beta dimers, and heme-free globin, but does not bind deoxyhemoglobin, myoglobin, heme, hemoglobin H, hemoglobin Barts, or free beta chains. The heterogeneity of the human alpha chain (with two major classes of alpha chains, denoted 1 and 2) gives rise to three common phenotypes, designated 1-1 (homozygous for class 1 alpha chains), 2-1 (heterozygous), and 2-2 (homozygous for class 2 alpha chains) respectively. All phenotypes have a similar beta chain. Phenotype 1-1 is a single molecular species with molecular weight of approximately 100,000, migrating electrophoretically as a single band. Phenotypes 2-1 and 2-2 are composed of multiple aggregates of the molecule and migrate as a series of discrete bands. The hemoglobin binding capacity of blood with a normal haptoglobin level varies slightly with the phenotype showing a hemoglobin binding capacity of 1.37 g/l for phenotype 1-1, 1.1 g/l for phenotype 2-1, and 0.8 g/l for phenotype 2-2. Normal human serum, with a haptoglobin concentration of 40–250 mg/dl has the capacity to bind approximately 0.4–2 g/l of hemoglobin. This is equivalent to the hemoglobin contained in approximately 20 ml of completely hemolyzed blood.

Methodology

There are several techniques to measure serum haptoglobin. 1) A common colorimetric technique is based on the principle that the hemoglobin–haptoglobin complex exhibits strong peroxidase activity in acid solution. 2) The hemoglobin binding capacity of haptoglobin may be measured by addition of excess hemoglobin followed by column chromatographic separation. 3) Spectrophotometric tests have been developed, one of which is based on the principle that haptoglobin protects cyanmethemoglobin from denaturization in acid solution. 4) Immunoprecipitation nephelometry is reported to be a rapid and sensitive methodology. 5) Radial immunodiffusion is technically simple, but may require knowledge of the patient's haptoglobin phenotype.

Clinical Utility

Serum haptoglobin has been considered a sensitive indicator of intravascular and to a lesser extent, extravascular hemolysis. Injection of hemoglobin into healthy human subjects results in a proportional decline in serum haptoglobin, reaching a
minimum in 8-10 hours. The half-life of the haptoglobin-hemoglobin complex is approximately 9-30 minutes. The haptoglobin level may also be useful in the preliminary work-up of megaloblastic anemias, which represent a form of "intramedullary hemolysis." In a recent study of haptoglobin using nephelometric techniques, Marchand found that all patients with autoimmune hemolytic anemias (both Coombs + and Coombs -) had severely reduced haptoglobin levels in the range of 0–25 mg/dl. All patients with mechanical hemolysis also had severely reduced levels. However, the author did not describe the reticulocyte count or peripheral smear morphology in these cases, making it difficult to evaluate the diagnostic contribution of the serum haptoglobin. Although the haptoglobin level appeared to be a sensitive indicator of hemolysis, 6% of patients with non-hematologic diagnoses and a significant fraction of patients with acute blood loss and iron deficiency anemia had levels in the low or severely reduced range. In clinical practice, the high prevalence of these later disorders would seriously detract from the predictive value of a low haptoglobin level.

With the peroxidase method for haptoglobin, Shinton found only 80% of patients with hemolytic disease (mainly congenital spherocytosis and autoimmune hemolytic anemia) had levels less than 40 mg/dl. There was low correlation between haptoglobin level and serum bilirubin. Sensitivity was lower in the diagnosis of megaloblastic anemias. Non-specific causes of reduced haptoglobin were common. Six percent of patients with iron deficiency and 21% of patients with recent hemorrhage had levels below 40 mg/dl. Low haptoglobin following blood loss may be due to the hemolysis of blood extravasated into tissues or body cavities.

**Limitations**

Approximately 2% of adult Caucasians and up to 30% of black Africans have congenital absence of haptoglobin. Interestingly, these patients do not have a clinically recognizable illness. Newborns and children have low or undetectable haptoglobin levels. Decreased haptoglobin is seen in liver disease: approximately 10% of patients with acute or chronic hepatitis have low haptoglobin levels. Post-transfusion intravascular hemolysis markedly reduces serum haptoglobin. However, in one study, 10% of patients showed a decrease in serum haptoglobin of greater than 25 mg/dl following an uneventful transfusion, possibly due to small amounts of hemolyzed blood in the donor bag. Massive transfusion might result in a greater depression of serum haptoglobin, even in the absence of a transfusion reaction.

Elevations of serum haptoglobin are observed in acute and chronic infection (acute phase reactant), malignancy, biliary obstruction, ulcerative colitis, myocardial infarction, and diabetes mellitus. In patients with untreated pernicious anemia having concomitant fractures, infection or malignancy, haptoglobin may be raised into the normal range.

**Conclusion**

Due to the high prevalence of many conditions associated with low haptoglobin (genetic deficiency, iron deficiency, recent hemorrhage, liver disease, unremarkable transfusion), a low serum haptoglobin must be interpreted with caution. The
haptoglobin determination cannot be recommended as a general screen for hemolysis. Specific indications for the test are not well defined. As part of the work up of a suspected hemolytic transfusion reaction, a low level would in many instances be non-contributory due to the numerous non-specific causes for a low haptoglobin. Even the prevalence of congenital haptoglobin deficiency is greater than the prevalence of acute intravascular hemolytic reaction on a typical transfusion service. High-normal levels probably rule out significant intravascular hemolysis. However, haptoglobin levels associated with alloantibody induced extravascular hemolysis have not been well described. The relatively long turnaround time of most methodologies further decreases the clinical utility of the test in emergent situations. For the diagnosis of post transfusion intravascular hemolysis, haptoglobin levels (if obtained) should probably be interpreted in conjunction with other clinical and laboratory findings, i.e., the peripheral smear, plasma and urine hemoglobin, review of cross-match records and patient identification, Coomb's test and serum bilirubin.

References


