TEST EVALUATION
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TEST

Hemoglobin F (fetal hemoglobin)

METHOD

Fetal hemoglobin is more resistant to alkaline denaturation than other hemoglobins. First, all hemoglobin species are converted to their corresponding cyanomethemoglobin forms. A hemolysate is then exposed to alkali followed by neutralization and precipitation of denatured adult hemoglobin with ammonium sulfate. The absorbance of the filtrate containing only the alkali-resistant fetal hemoglobin is measured and compared to the absorbance of the total hemolysate. Results are expressed as the fraction (%) of total hemoglobin that is resistant to denaturation.

CLINICAL APPLICATIONS

Hemoglobin F (HbF) is the main red cell hemoglobin component in fetal development. At birth, HbF makes up between 60% and 90% of red cell hemoglobin. This usually decreases to the adult range of 0-2% at 6 months of age, but this varies from individual to individual.

Elevated levels of HbF, usually between 2-5%, have been reported in some cases of hereditary spherocytosis, leukemia, aplastic anemia, megaloblastic anemia, and carcinoma metastatic to bone.

Approximately 50% of patients with β-thalassemia trait will have HbF levels of 2-5%. In patients with homozygous β-thalassemia, HbF is almost always markedly increased, usually reaching levels of 15-100%.

Homozygotes for HbS may have no fetal hemoglobin or levels as high as 20%. Heterozygotes for HbS have normal levels of HbF.

When fetal hemoglobin reaches levels of 15% in patients with no other hematologic disorder, heterozygosity for hereditary persistence of fetal hemoglobin (HPFH) should be suspected. This is an asymptomatic disease found primarily in blacks and Greeks. Although the findings in these two ethnic groups are similar, they are thought to be of different genetic origins.

Homozygous HPFH is rare and found only in blacks. These people have 100% HbF but no anemia. They may have mild hypochromia and microcytosis.

CLINICAL AND TECHNICAL LIMITATIONS

It is important to differentiate heterozygous HPFH from heterozygous β-thalassemia. In HPFH, the sum of beta- and gamma-chain production is equal to alpha-chain production. In thalassemia, there is a selective decrease in beta-chain
synthesis and an associated hypochromia and microcytosis. HbF is evenly distributed among all red cells in HPHF. In thalassemia, HbF is heterogeneously distributed. This can be determined by a Kleihauer-Betke test. Hemoglobin F is precipitated and fixed in red blood cells on a peripheral smear. Homogeneous versus heterogeneous red cell populations can be determined.

The alkali denaturation method is fast and easy to perform. A significant disadvantage is that HbF is not entirely resistant to alkaline denaturation and can be underestimated by this method.

Another potential problem is that HbF may be absorbed by filter paper during filtration. Choice of an appropriate grade of filter paper may minimize this problem.

Other methods for measuring fetal hemoglobin include column chromatography, radioimmunoassay, an enzyme-linked immunoabsorbent assay (ELISA) and radial immunodiffusion.

REFERENCES
