Prenatal Diagnosis of Hemoglobinopathies by Using Polymerase Chain Reaction and Allele-Specific Oligonucleotide Probes, D. Crisan,1 W. F. Diven,1 K. Hartle,1 P. T. Osborne,2 and L. Mason2 (Depts. of Pathol., 1 Univ. of Pittsburgh, Pittsburgh, PA 15213, and 2 Univ. of Tennessee, Memphis, TN 38163)

The prevalence of hemoglobinopathies, their widespread distribution, and their potential for clinical severity make prenatal diagnosis and testing for carriers of these disorders important public health issues. Although current hematologic tests are adequate for carrier screening, prenatal diagnosis during the first trimester is possible only by using molecular pathology techniques.

Direct analysis of fetal DNA for identification of point mutations provides a high degree of diagnostic specificity. The analysis must be highly sensitive, to permit utilization of the small amounts of fetal DNA obtained by amniocentesis orchorionic villi sampling, procedures that are safer than sampling fetal blood. The requirements for specificity have been met by most hybridization techniques, but the need for high sensitivity has been a procedural limitation. The sensitivity has been increased by several orders of magnitude by in vitro enzymatic amplification of the genomic beta-globin gene sequences of interest by the polymerase chain reaction (PCR).

In this study we compared (a) effectiveness of the PCR amplification of a beta-globin sequence from purified vs cell-lysate DNA, and (b) utilization of the amplified products by hybridization with allele-specific oligonucleotide (ASO) probes vs restriction endonuclease analysis. Using two different pairs of primers in the PCR technique, we amplified two target beta-globin sequences. Both sequences encompassed the point mutations responsible for the beta and beta alleles, which correspond to hemoglobins S and C, and the normal sequence of the beta allele, corresponding to hemoglobin A. Depending on which pair of primers we used, we obtained a 110-bp or 725-bp product (1-3). The 110-bp product was used for ASO hybridization; the larger product was necessary for restriction endonuclease digestion. We wanted to determine whether a rapid procedure with use of cell-lysate DNA and restriction endonuclease analysis would provide the same sensitivity and specificity as the comparison method, utilizing purified DNA and ASO hybridization, for clinical application in the prenatal diagnosis of hemoglobinopathies (hemoglobins S and C).

Purified DNA from peripheral blood leukocytes was obtained from six normal volunteers and from 17 patients with hemoglobinopathies: seven with hemoglobin SS, five with hemoglobin AS, three with hemoglobin SC, and two with S-beta thalassemia. We obtained fetal DNA from 12 random amniotic-fluid samples, and applied the PCR amplification to the purified DNA or crude amniotic-cell lysates.

For all patients, hybridization of the 110-bp product with ASO probes for beta, beta, and beta gave hybridization patterns that were consistent with the phenotype diagnoses based on results of the standard diagnostic procedure, hemoglobin electrophoresis. In the fetal DNA samples, amplification and hybridization were equally successful with both types of DNA preparation. The hybridization patterns were consistent with a normal beta-globin genotype for all 12 fetal samples and for the six normal controls.

In the study with restriction endonuclease, we obtained Cvn I digestion patterns of the 725-bp amplification product (from the same starting DNA preparations and cell sources as the 110-bp product). In all cases of sickle-cell anemia and sickle-cell trait, as well as in the normal controls and the fetal DNA samples, the digestion patterns were consistent with the hybridization patterns. Double heterozygous states, such as hemoglobin SC and S-beta thalassemia, need further analysis with a battery of restriction endonucleases or ASO probes for diagnosis. This becomes necessary when analysis of parental DNA demonstrates the possibility of a double heterozygous fetus.

to demonstrate the diagnostic capability of these procedures, we coded and arranged the samples in simulated family sets involving pregnancies at risk for sickle-cell anemia, as previously done by Saiki et al. (4). DNA amplification was equally successful whether we used purified DNA or DNA from cell lysates. Both ASO hybridization and restriction endonuclease digestion of amplified products resulted in identical genotypes for all subjects, consistent with the known clinical and hematological diagnoses.

We conclude that combining PCR amplification of beta-globin target sequences with analysis of the Cvn I digestion patterns results in the same specificity and sensitivity as the method involving ASO probe hybridization. Restriction endonuclease digestion is more advantageous for clinical application because it offers a rapid diagnosis in pregnancies at risk for sickle-cell anemia, i.e., in 6-8 h, as compared with one to two weeks for the ASO hybridization.

References


Real-time monitoring of glucose or other constituents in blood is hindered by the invasive nature of random checks, by the time-consuming analytical processes involved (mostly enzymatic reactions), and the requirement for more or