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PRENATAL DIAGNOSIS OF SICKLE CELL ANEMIA USING PCR AND RESTRICTION ENZYME Dde I*

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Prenatal diagnosis of sickle cell anemia was carried out in four fetuses using DNA technology. Fetal chorionic villus specimen were obtained at the 10th week of pregnancy from women at risk of giving birth to children with sickle cell anemia. Whole cellular DNA was obtained and the part of the DNA presumed to have a mutation increased after PCR was performed. After the application of Dde I restriction enzyme, mini gel electrophoresis was performed. The study of the electrophoretic patterns of the DNA indicated that one of the four fetuses was unaffected, one was a carrier and the remaining two were affected. *Key words: prenatal diagnosis, sickle cell anemia, polymerase chain reaction.*

Sickle cell anemia is prevalent in Africa and in blacks of African origin. It is also seen in some Mediterranean countries[1]. In some regions of Turkey, the prevalence of sickle cell anemia is reported to be around 15 percent[2].

There is high mortality and morbidity associated with this disease. At present there is no known cure for sickle cell anemia. Eradication of this disease can only be accomplished by genetic counselling and prenatal diagnosis. Prenatal

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diagnosis of sickle anemia began in the early seventies by the use of fetoscopy. In vitro globin synthesis analysis was replaced by DNA analysis in the beginning of the eigthies[3-5].

Recent developments in DNA technology has made possible the use of this technique in small laboratories, which is a safe, sensitive, reliable and reproducible method [6]. Below is a report of the preliminary experiences we had in our center in diagnosing sickle cell anemia in which the polymerase chain reaction (PCR) and restriction enzyme Dde I methods were employed.

Materials and Medhods

Chorionic villus biopsies were obtained from four women at the 10th week of pregnancy who were at risk of giving birth to children with sickle cell anemia.

The fetal and maternal parts of the chorionic villus specimens were separated under an inverted tissue microscope and the fetal chorionic villus tissue was placed in medium 199, obtaining cellular DNA[7]. PCR was performed by using synthetic oligoprimers (GGC, CAA, TCT, ACT, CCC, AG and ACA, TCA, AGG, GTC, CCA, TAG, AC) as templates close to the 5' and 3' ends of the mutant part of the B gene[8]. Amplified DNA products were digested with the specific restriction enzyme Dde I. The digested products were anlyzed in agarose gel containing 2% Nusieve agarose and 1% agarose (FMC Bio Products) in the presence of ethidium bromide. The electrophoretic pattern was examined under ultraviolet light. Restriction enzyme Dde I recognizes the CTAAG pattern sites and yields two bands of 150 and 201 bp in the part of the normal DNA obtained after carrying out the above procedure. In sickle cell disease, the presence of a CTGAG-CTGTG mutation removes the restriction recognition part, therefore, only one band of 351 pb is obtained from mutant DNA[8,9].

Our study revealed that one of the four fetuses was unaffected, one was a carrier and the remaining two had sickle cell anemia (Table I; Fig. 1).

Table I: Prenatal Diagnosis of Sickle Cell Anemia

	Fragment (kb) (Dde I)	Genotype
1. Fetus	351	SS
2. Fetus	351	SS
3. Fetus	351, 201, 150	SA
4. Fetus	201, 150	AA

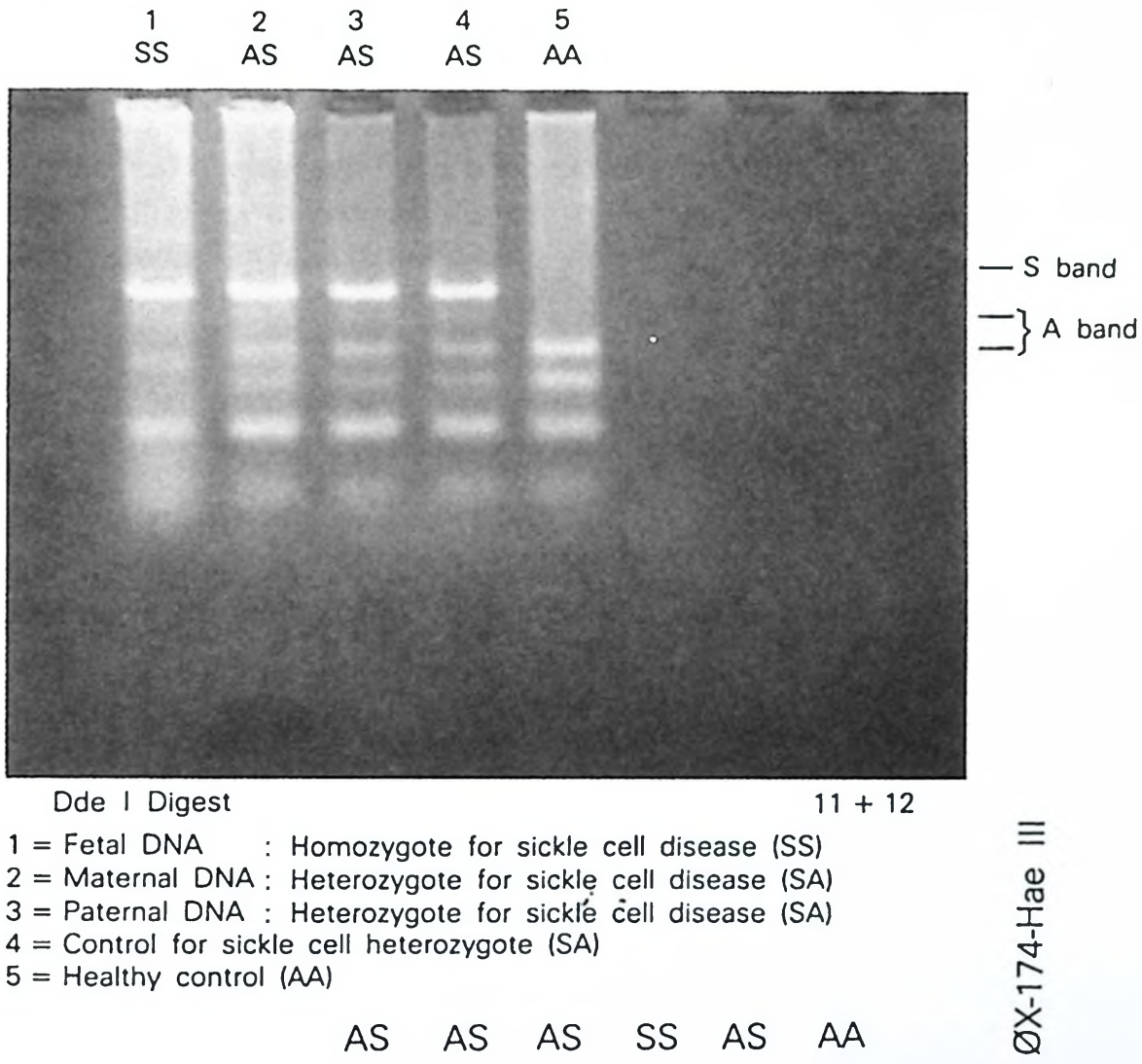

Fig. 1: Ethidium bromide stain of Dde I digested amplified DNA product from individuals with sickle cell trait (AS), sickle cell anemia (SS), normal β-globin genes (AA), and marker (ØX-174-Hae III).

Discussion

Hemoglobinopathies commonly occur in Turkey and prenatal diagnoses using fetoscopy and in vitro globin synthesis analysis have been available in our center for the last eight years[4, 10-12]. However, the total number of women with at-risk pregnancies who seek prenatal diagnosis is unexpectedly low. Fetoscopy is usually performed between the 18-19 week of pregnancy, and would-be mothers probably do not want to wait that long for a prenatal diagnosis. Besides, termination of pregnancy at this stage also creates medical and psychological problems. We hope that prenatal diagnosis in which DNA technology is employed, will encourage more women with at-risk pregnancies and also other would-be mothers to seek prenatal diagnoses. Several DNA methods are used to detect a sickle cell mutation, but all require PCR[8]. Our study indicates that mutations which cause changes in recognition sites of restriction enzymes can easily be detected.

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