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The Use of Early Simultaneous Percutaneous Umbilical Blood Sampling (PUBS) and Amniocentesis for Prenatal Fragile X Chromosome Diagnosis

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Abstract

Early simultaneous percutaneous umbilical blood sampling (PUBS) and amniocentesis for prenatal diagnosis were undertaken for the first time in a 17-week gestation fetus at risk for the fragile X [fra (X)] syndrome. Metaphase spreads from 300 fetal lymphocytes were examined within 5 days following PUBS, while approximately 5 weeks were required for the analysis of 148 amniocytes. The chromosomes were interpreted as normal (46,XX) and the fetus as fragile X-negative at the time of prenatal diagnosis. This was cytogenetically confirmed after delivery of a healthy term female infant. Our results suggest that early PUBS may become a useful adjunct to amniocentesis because of shorter culture time and earlier diagnosis.

Keywords

Martin-Bell syndrome; fragile X syndrome; prenatal diagnosis; percutaneous umbilical blood sampling (PUBS); 17-week-gestation fetus

INTRODUCTION

The fragile X [fra (X)] or Martin-Bell syndrome is the second-most-common cause of mental retardation after Down syndrome. While Down syndrome is usually a sporadic event, the fra (X) syndrome is transmissible and is the commonest form of Mendelian mental retardation.

Prenatal diagnosis of the fra (X) syndrome has been accomplished by cytogenetic analysis of amniotic cells obtained by amniocentesis [Jenkins et al., 1981; Shapiro et al., 1982; Shapiro and Wilmot, 1984; Oberlé et al., 1985]; fetal lymphocytes obtained by fetoscopy [Webb et al., 1981]; and trophoblastic cells obtained by chorionic villus biopsy [Tommerup et al.,

1985]. These various methods for obtaining fetal cells are not without risk to the fetus. Fetal risks for amniocentesis, fetoscopy, and chorionic villus biopsy are about 0.3%, 4–5%, and 1%, respectively [Nicolaidis, 1984; Daffos et al., 1985; Jackson, 1986].

CLINICAL REPORT

Webb et al. [1983] proposed that fetal blood examination should enable rapid and accurate prenatal diagnosis of the fra (X) syndrome. In experienced hands, the fetal risk of 1% for PUBS is comparable to that of chorionic villus biopsy [Daffos et al., 1985]. Therefore, to compare the efficacy of fetal lymphocyte versus amniotic cell chromosome analysis, we performed early simultaneous percutaneous umbilical blood sampling (PUBS) and amniocentesis on a 17-week-gestation fetus at risk for the fra (X) syndrome. The mother's lymphocytes grown in Medium 199 and fluorodeoxyuridine (FUdR) showed a 4% fra (X) chromosome expression, while her brother affected with the fra (X) syndrome had 24% fra (X) expression. She has 6 affected male cousins whose fra (X) chromosome expression ranged from 4% to 36% in Medium 199 and FUdR. She also has 2 normal sons, ages 6 years and 8 months, one of whom had been evaluated prenatally to rule out the fra (X) syndrome. We have also successfully analyzed lymphocytes obtained by PUBS from 6 fetuses without a history of fra (X) syndrome for determination of fragile site expression. The analysis of rare (folate sensitive) and common fragile sites was also undertaken to monitor the fragile site induction system [Wilmot and Shapiro, 1986; Lele et al., 1986] (Table I). Recording of fragile sites may be useful for confirmation of the fragile site induction system but additional research is needed to determine which sites are most reliable.

RESULTS AND DISCUSSION

At 17 weeks of gestation, genetic amniocentesis and PUBS were undertaken without complications. Approximately 1.5 ml of fetal blood was obtained from the umbilical cord at the placenta insertion site by ultrasound guided PUBS and 30 ml of amniotic fluid by amniocentesis. Amniotic cells and fetal lymphocytes were cultured in either RPMI 1640 or Medium 199 and FUdR. Two hundred fetal blood cells grown in Medium 199 alone and an additional 100 cells grown in Medium 199 and FUdR (10^{-7} M) were examined within 5 days of PUBS. One hundred forty-eight metaphases were analyzed from amniocytes by the fifth week following amniocentesis from 2 separate laboratories. Seventy-five cells were cultured in RPMI 1640 medium and changed to Medium 199 and FUdR (10^{-7} M) during the last 24 hours, while 73 cells were cultured in a second laboratory in RPMI 1640 medium and FUdR (10^{-7} M) added during the last 24 hours before harvesting. These amniotic fluid cell results were considered suboptimal since at least 150 cells from 3 different cytogenetic methods are usually analyzed. All chromosomes were stained with Giemsa and subsequently G-banded to identify the specific chromosomes, but no fragile X expression was observed in either the fetal lymphocytes or amniotic cells. The chromosomes were interpreted as normal (46,XX).

The pregnancy progressed without complications and a healthy term girl weighing 2.5 kg was delivered vaginally. Peripheral blood was obtained, and 103 lymphocytes grown in RPMI 1640 medium and FUdR were analyzed. No Xq27.3 fragile site was observed which

confirmed the negative fra (X) findings at the time of prenatal diagnosis. DNA studies are also underway to identify the fra (X) chromosome in several members of this family.

To our knowledge this is the first report in this country of the utility of PUBS at 17-weeks gestation for prenatal diagnosis of the fra (X) syndrome. Additional studies will be required, but early PUBS may become a useful adjunct to amniocentesis because of shorter culture time (4 days versus 2 to 6 weeks) and earlier diagnosis.

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TABLE I

Fragile X Expression in Control Fetuses and the Present Case

	Control subjects						Present case	
	1	2	3	4	5	6	Lymphocytes	Amniocytes
Weeks of gestation	21	23	23	27	28	28	17	
No. of cells analyzed	55 ^{a,c}	50 ^{a,c}	50 ^{a,c}	50 ^{a,c}	50 ^{a,c}	50 ^{a,c}	300 ^{b,c}	148 ^d
Fragile X expression (%)	0	0	0	0	0	0	0	0
Expression (%) & location of other fragile sites	2	2	0	0	2	0	2	0
	16q23	16q23			10q23		10q23 Xp22 16q23	

^aCells grown in Medium 199 and FUDR.^b200 cells in Medium 199
100 cells in Medium 199 and FUDR.^cFetal lymphocytes obtained by PUBS.^dCombined No. from two laboratories.