Prenatal diagnosis of β-thalassaemia by coelocentesis

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Coelomic fluid and placental tissue were obtained from four women undergoing termination of pregnancy at 7–9 weeks gestation for psychological reasons. All four women and their partners were known carriers of β-thalassaemia and DNA analysis in their blood identified the mutation carried by each of them. Allele-specific polymerase chain reaction and denaturing gradient gel electrophoresis techniques were used to detect and identify the mutations in the DNA extracted from the coelomic cells and placental tissue. Three fetuses were found to be carriers of either the paternal or maternal mutation, while one was found to be affected by β-thalassaemia. There was concordance in the results obtained from the chorionic villi and coelomic cells. Amplification of the apolipoprotein B gene variable number tandem repeats (VNTR), in the DNA of the coelomic cells showed normal segregation of alleles in the fetuses, thus excluding maternal contamination. The results of this study demonstrate that coelocentesis may be a reliable alternative technique for the diagnosis of β-thalassaemia from as early as 7 weeks gestation.

Key words: β-thalassaemia/coelocentesis/prenatal diagnosis

Introduction

First trimester prenatal diagnosis can be performed by chorionic villus sampling (CVS) or amniocentesis. A recent study comparing the two techniques has reported that the risk of fetal loss from amniocentesis at 10–13 weeks gestation is ~3% higher than that following CVS (Nicolaides et al., 1994). However, CVS may be associated with a higher risk of fetal loss compared with second trimester amniocentesis (Canadian Collaborative CVS–Amniocentesis Clinical Trial Group, 1989; Medical Research Council Working Party, 1991). Additionally, there is controversial evidence that CVS, when performed before 11 weeks gestation, could be the cause of transverse limb abnormalities and oromandibular hypoplasia (Firth et al., 1991; Froster and Jackson, 1996).

An alternative technique that may prove to be effective for early prenatal diagnosis is coelocentesis. During the first trimester of pregnancy the amniotic sac is surrounded by the exocoelomic cavity which is a derivative of the extraembryonic mesoderm. Several studies have reported successful aspiration of coelomic fluid in nearly all cases at 6–10 weeks gestation. Examination of the fluid demonstrated that although cells from the exocoelomic cavity cannot be successfully cultured, embryonic genetic material can be analysed using fluorescence in-situ hybridization (FISH) and polymerase chain reaction (PCR) techniques (Jurkovic et al., 1993, 1995; Findlay et al., 1996).

The aim of this study is to explore the potential use of coelocentesis in the prenatal diagnosis of other genetic disorders such as β-thalassaemia.

Materials and methods

Coelocentesis was performed in four singleton pregnancies at 7–9 weeks gestation, immediately before surgical termination for psychological reasons at Ioannina University Hospital, Greece. All four women and their partners were known carriers of β-thalassaemia. Ethical approval for this study was obtained from the Hospital Research committee and the women gave written consent.

Coelocentesis

The external genitalia and the vagina were carefully cleansed with an antiseptic solution and the 5MHZ ultrasound transducer (Toshiba SSA-220A, Tokyo Japan) was covered with a sterile rubber. Subsequently, a transvaginal scan was performed for the measurement of the fetal crown–rump length and fetal heart rate (using the M-mode of the machine), identification of the amniotic membrane, coelomic space and yolk sac, and diagnosis of any uterine abnormalities. After the introduction of general anaesthesia, a 20 G needle attached to the transducer was introduced into the coelomic cavity and coelomic fluid (1–2.5 ml) was aspirated. Care was taken to avoid puncture of the amniotic membrane or the yolk sac and the tip of the needle was visualized constantly. Immediately after the procedure the fetal heart rate was measured again and suction termination was performed. Placental tissue was collected after the termination in every case and peripheral blood was obtained from all women and their partners for the identification of the β-globin gene mutations.

DNA extraction

Coelomic cells were isolated after centrifugation of the coelomic fluid and the removal of the supernatant. The cell pellet was washed twice in a buffer containing 10 mM Tris–HCl, 50 mM KCl, 1.5 mM MgCl₂ and 0.001% gelatine, resuspended in 25 μl of the same buffer, and placed in boiling waterbath for 20 min. The samples were centrifuged briefly and were used in 2 μl aliquots of the supernatant for PCR.
Figure 1. β-globin gene analysis by (A) denaturing gradient gel electrophoresis (DGGE) and (B) amplification refractory mutation system (ARMS) of case no 1. Lane 1 represents the father, lane 2 the mother, lane 3 the placental DNA, lane 4 the coelomic cell DNA, and lane 5 a normal control without the IVS1–110 mutation. M = molecular weight marker (φx 174/HaeIII). In (A), the additional bands in lanes 1 and 2 are heteroduplexes of the normal and mutant alleles which normally appear in heterozygotes of β-thalassaemia. In (B), the two lower bands represent ARMS–polymerase chain reaction products, the lowest being specific for the IVS1–110 mutation.

Table I. Fetal crown–rump length (CRL), amount of coelomic fluid aspirated (COE), parental and fetal β-thalassaemia mutations and fetal heart rate before (FHR-B) and after (FHR-A) coelocentesis

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gestational age</th>
<th>CRL (mm)</th>
<th>COE (ml)</th>
<th>Father</th>
<th>Mother</th>
<th>Fetus</th>
<th>FHR-B</th>
<th>FHR-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 w 5 d</td>
<td>22.3</td>
<td>1.5</td>
<td>IVS1–110/N</td>
<td>IVS1–110/N</td>
<td>IVS1–110/IVS1–110</td>
<td>181</td>
<td>184</td>
</tr>
<tr>
<td>2</td>
<td>8 w</td>
<td>16</td>
<td>1</td>
<td>IVS1–6/N</td>
<td>IVS1–110/N</td>
<td>IVS1–6/N</td>
<td>174</td>
<td>171</td>
</tr>
<tr>
<td>3</td>
<td>7 w</td>
<td>12.3</td>
<td>2.2</td>
<td>IVS1–110/N</td>
<td>CD–39/N</td>
<td>IVS1–110/N</td>
<td>142</td>
<td>144</td>
</tr>
<tr>
<td>4</td>
<td>7 w 4 d</td>
<td>14</td>
<td>2.5</td>
<td>CD–39/N</td>
<td>IVS1–1/N</td>
<td>IVS1–1/N</td>
<td>156</td>
<td>156</td>
</tr>
</tbody>
</table>

w = weeks; d = days; N = normal allele

DNA was also extracted from placental tissues and parental peripheral blood by the standard salt extraction method.

Amplification of the β-globin gene

Two PCR approaches were used for the identification of mutations from the coelomic cells, peripheral blood and placental DNA. The first, the amplification refractory mutation system (ARMS), is specific for the mutant alleles, and the second, denaturing gradient gel electrophoresis (DGGE; Figure 1) is a screening method for mutations (Losekoot et al., 1990; Old et al., 1990). The primers used of the ARMS were originally described by Old et al. (1990). Both cycling protocols were adjusted to 40 cycles, with the first 10 cycles having extended annealing (3 min) and elongation (3 min) intervals. Amplification of the apolipoprotein B gene variable number tandem repeats (VNTR), for the exclusion of coelomic cell contamination from maternal tissues was also performed (Decorte et al., 1990).

Results

Coelomic fluid was obtained with the first attempt in all four cases and no major changes were observed in the fetal heart rate before and after the procedure. Detection of the parental mutations was successful in the coelomic DNA in all cases. One fetus was found to be affected by the disease and the remaining three were carriers of β-thalassaemia (Table I). There was concordance in the results from the coelomic fluid and placental tissue. Normal segregation of alleles of the parents and the fetus were identified with the use of VNTR (results not shown). The fetal crown–rump length, fetal heart rate, gestational age, amount of coelomic fluid aspirated and parental mutations are shown in Table I.

Discussion

The diagnosis of β-thalassaemia is the most common indication for first trimester invasive prenatal diagnosis, in Greece and other Mediterranean countries, because up to 10% of the population are carriers of an abnormal gene (Loukopoulos et al., 1985). The serious implications of the disease for both the patients and their families, in combination with the high chance (25%) of having an affected baby when both parents are carriers of the disease, makes early prenatal diagnosis essential. Therefore, CVS is widely used after 11 weeks gestation, in order to avoid the limb reduction syndrome (Firth et al., 1991). Alternatively, amniocentesis is performed during the second trimester, while cordocentesis is used in those patients presenting beyond 20 weeks gestation.

The results of this study have demonstrated that coelocentesis could be used for the prenatal diagnosis of β-thalassaemia. Similarly, recent reports have demonstrated that PCR can successfully be used for the determination of fetal sex from coelomic cells and nested PCR can be used for the diagnosis of sickle cell anaemia. However, Jurkovic et al. (1995) have stressed the need for stringent laboratory conditions in order to avoid the possibility of contamination from maternal cell DNA. Although in our cases there was concordance in the
results from the coelomic cells and those of the placenta, and therefore maternal contamination is unlikely, the demonstration of normal fetal complements of alleles in the coelomic cells, from both parents with the use of VNTRs, excludes the possibility of maternal contamination when prenatal diagnosis is performed from coelomic cells only.

Coelocentesis cannot be used in prenatal diagnosis at present since the risks associated with this technique are uncertain because it has not been carried out in continuing pregnancies. However, this procedure does not involve puncture of the amniotic membrane or the placenta and therefore may be safer than early amniocentesis or CVS (Jurkovic et al., 1993). Moreover, a recent study (Makrydimas et al., 1997) demonstrated that coelocentesis is not associated with changes in maternal serum α-feto-protein and therefore significant fetal bleeding following this procedure is unlikely. In the event that coelocentesis proves to be safe for the fetus, prenatal diagnosis of β-thalassaemia would be possible from at least 7 weeks gestation reducing the anxiety of the parents by 1 month or more and making the possible termination of the pregnancy easier and less traumatic. In addition, the earlier diagnosis of the homozygotes for the disease could permit the injection of stem cells into the coelomic cavity, for the induction of tolerance and chimaerism and possible treatment of this condition (Edwards et al., 1995).

References


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