Blood chimerism in monochorionic twins conceived by induced ovulation: Case report

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A case of monochorionic twin boys delivered at 34 weeks of gestation following induced ovulation with clomiphene is described. One twin was typed as blood group AB and the other as B. Flow cytometry showed blood group chimerism. DNA polymorphism analysis of peripheral lymphocytes and hair root cells showed that the chimerism was confined to the blood cells and they were dizygotic.

Key words: blood chimerism/dizygotic twins/induced ovulation/monochorionic placenta

Introduction

Souter et al. (2003) reported that monochorionicity does not necessarily mean monozygosity. In their case, monochorionic twins, the product of IVF, showed discordant sex, chromosomal chimera in peripheral blood lymphocytes and normal karyotype in cultured skin fibroblasts from each twin. We report monochorionic dizygotic twins conceived by induced ovulation who were the same gender with confined blood chimerism.

Case report

A 27 year old primigravida woman who conceived by clomiphene citrate delivered twin boys at 34 weeks of gestation by Cesarean section. An ultrasound study at 12 weeks of gestation had shown that she had had a monochorionic placenta. Twin A was a boy with birthweight of 2002 g and twin B was also a boy with weight of 2132 g, and both twins were ventilated because of respiratory distress syndrome. A routine blood group typing using anti-A and -B sera at birth showed twin A was blood group ‘AB’ and twin B was ‘B’. Their father was ‘AO’ and mother was ‘AB’. A pathological examination of placenta revealed monochorionic diamniotic placenta which contradicted our understanding that monochorionic twins should be monozygotic.

Materials and methods

In order to elucidate the contradiction of monochorionicity and different blood groups in twins, with the oral consent from the parents, various blood group typing for the twins’ red cells was undertaken with commercially available anti-sera for the following blood group antigens: Rh, MNS, Xg, P, Kell, Lutheran, Duffy, Kidd and Lewis. Flow cytometry of the erythrocytes was also undertaken at 3 months of age. To demonstrate dizygosity, HLA class II DRB1 antigen of lymphocytes and DNA polymorphism of peripheral lymphocytes and hair root cells with STR Systems (GenePrint fluorescent STR systems; Promega Co., Madison, WI, USA) were also analysed.

Results

Blood groups except ABO groups were the same in both twins (Table I), although it was possible that very subtle blood group chimerism was not detected because the test was haemagglutination for anti-sera, and it could easily miss a small amount of chimeric blood group. Flow cytometry showed blood group chimerism, i.e. twin A had AB (88%)/B (12%) blood group chimera and twin B had B (99%)/AB (1%) (Table I). HLA typing was identical in both twins. However, DNA polymorphism analysis showed that five loci out of nine were different in peripheral lymphocytes and hair root cells between the twins. Out of those five different loci, two minor bands of allele which twin B had were detected in peripheral lymphocytes of twin A (Table II).

Discussion

Monochorionicity had been a characteristic of identical twins until Souter et al. (2003) reported monochorionic sex-discordant twins with blood cell chimerism. Although Nylander and Osunkoya (1970) reported partial monochorionic placenta of sex-discordant twins, the possibility of the existence of monochorionic dizygotic twins has not been paid much attention because of the deficiency of cytogenetic studies in their report. In the report by Souter et al. the twins were discordant in gender and showed chimerism of karyotype and genotyping of peripheral lymphocyte. Cultured skin fibroblasts showed normal karyotype and different DNA markers between twins (Souter et al., 2003). In 2005, Miura and
Blood group typing in each twin

Table I. Blood group typing in each twin

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Twin A</th>
<th>Twin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh</td>
<td>ccDDe</td>
<td>ccDDe</td>
</tr>
<tr>
<td>MNS</td>
<td>MNss</td>
<td>MNss</td>
</tr>
<tr>
<td>Xg</td>
<td>Xg(a−)</td>
<td>Xg(a−)</td>
</tr>
<tr>
<td>P</td>
<td>p</td>
<td>p</td>
</tr>
<tr>
<td>Kell</td>
<td>K−k+</td>
<td>K−k+</td>
</tr>
<tr>
<td>Lutheran</td>
<td>a+b+</td>
<td>a+b+</td>
</tr>
<tr>
<td>Duffy</td>
<td>a+b−</td>
<td>a+b−</td>
</tr>
<tr>
<td>Kidd</td>
<td>a+b+</td>
<td>a+b+</td>
</tr>
<tr>
<td>Lewis</td>
<td>a+b+</td>
<td>a+b+</td>
</tr>
<tr>
<td>ABO</td>
<td>AB (88%)/B (12%)</td>
<td>B (99%)/AB (1%)</td>
</tr>
</tbody>
</table>

*Different loci between each twin.

In parentheses: minor band of allele found only in peripheral lymphocytes. NA = not available.

Niikawa (2005) reported five Japanese cases of monochorionic dizygotic twins other than Souter’s case. All cases were associated with assisted reproductive technology; three cases with IVF, one with induced ovulation and intrauterine insemination and one with ICSI. All cases were discordant in gender and showed chimerism of karyotype in peripheral lymphocytes. In one case, cultured skin fibroblasts showed normal karyotype and in another case a different genotype in lymphocytes and skin was found. In another case, only blood group chimerism was examined. Different from previous cases, our twins were concordant in gender and a doubt about monochorionicity was entertained due to the blood group discordancy. Flow cytometry and polymorphism study showed that each twin carried two populations of erythrocytes and lymphocytes. Blood chimerism is caused by placental anastomoses which are very common in monochorionic placenta and allows reciprocal intrauterine transfusion of blood stem cells. Fetal immunological immaturity allows transfused stem cells to proliferate in the other’s bone marrow and results in the coexistence of two populations of blood cells in one individual (van Dijk et al., 1996).

Explanations for monochorionic placentation in dizygotic twins have been speculated upon. Nylander and Osunkoya (1970) proposed the possibility of fusion of chorions early in pregnancy with subsequent degeneration of fused chorions, but there has been no report to substantiate this possibility. The other possibility is fusion of two blastocysts before implantation (Souter et al., 2003). This possibility is supported by the study indicating fusion of two blastocysts could be induced in vitro under certain conditions (Tarkowski and Wojewodzka, 1982). Redline commented in the same issue of the Journal as Souter et al. that fusion of blastocysts had been considered to be unlikely since blastocysts are surrounded by the apical surface of a tight epithelial layer. Nevertheless he speculated that there may be a very short period in which two pre-blastocysts are able to fuse (Redline, 2003), although in Souter’s case, oocytes were cultured to the blastocyst stage and transferred in the uterus. Miura and Niikawa (2005) stated that assisted hatching, simultaneous embryo transfer, the use of fertilized oocytes that have developed to the blastocyst stage, and cell culture procedures that lead to changes of the nature of cell surface, may all increase the chance of a cell fusion.

Another possibility is binovular follicle fertilization. A binovular follicle, which has two oocytes surrounded by single zona pellucida, has been reported in humans who had been given gonadotrophins (Papadaki, 1978). One or both oocytes in a binovular follicle have been reported to be fertilized but there has been no report of establishment of a viable pregnancy (Zeilmaker et al., 1983; Ben-Rafael et al., 1987; Ron-El et al., 1990; Safran et al., 1998; Vicdan et al., 1999). In our case, twins were conceived with clomiphene without artificial manipulation. Therefore it is conceivable that a binovular follicle induced by a high level of gonadotrophin due to clomiphene leads to clonal fusion of two embryos and monochorionic placentation with vessel anastomoses and results in blood chimerism.

Whatever the underlying mechanism may be, when discordant sex or different blood groups which seem incompatible with monochorionicity do not exist, monochorionic dizygotic twins would be easily mistaken as monochorionic monozygotic twins. Although monochorionic placenta is not common in assisted reproduction twins, determination of monoygosity in monochorionic twins conceived by assisted reproduction should be done cautiously.

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References


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