Full karyotyping, rapid aneuploidy diagnosis or both when invasive prenatal testing is performed for diagnosis of thalassaemia?

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A retrospective study was performed to compare the detection rate of chromosomal abnormalities by different approaches of full karyotyping, rapid aneuploidy diagnosis (RAD) or both when invasive prenatal testing is performed for diagnosis of thalassaemia. The karyotype results of 1120 prenatal samples obtained from thalassaemia couples from January 1985 to December 2002 in a referral centre for prenatal diagnosis were studied. The detection rate of chromosomal abnormalities by four different approaches were compared: (i) karyotyping for all samples; (ii) RAD (21,18,13,X,Y) for all samples; (iii) RAD for all samples + karyotyping for cases with ultrasound abnormalities; and (iv) RAD (21,18,13) for all + RAD (X,Y) for cases with ultrasound abnormalities consistent with Turner syndrome + karyotyping for cases with ultrasound abnormalities. Normal karyotypes were found in 1103 samples (98.5%). There were 17 cases (1.5%) of chromosomal abnormalities: four cases (0.36%) were clinically significant, eight cases (0.7%) were of borderline clinical significance and five cases (0.44%) were not confirmed by subsequent prenatal or postnatal tests. The incidences of autosomal (7/1120 = 0.63%) and sex chromosomal (5/1120 = 0.45%) abnormalities were not higher than those (0.41 and 0.22%, respectively) from newborn surveys (Hook and Hamerton, 1977) (P = 0.398 and 0.216, respectively). Approach 1 would detect all 17 chromosomal abnormalities. Approach 2 would detect three of four clinically significant chromosomal abnormalities but not detect six of eight chromosomal abnormalities of borderline clinical significance and three of five chromosomal abnormalities not confirmed by subsequent prenatal or postnatal tests. Approach 3, in addition, would be able to detect all four clinically significant chromosomal abnormalities. Approach 4 would detect all four clinically significant chromosomal abnormalities but would not detect seven of eight chromosomal abnormalities of borderline clinical significance and four of five chromosomal abnormalities not confirmed by subsequent prenatal or postnatal tests. RAD (21,18,13) for all + RAD (X,Y) for cases with ultrasound abnormalities consistent with Turner syndrome + karyotyping for cases with ultrasound abnormalities seemed to be the best approach for the detection of chromosomal abnormalities when invasive prenatal testing is performed for diagnosis of thalassaemia.

Key words: rapid aneuploidy diagnosis, karyotyping, thalassaemia

Introduction

Thalassaemia is common in Hong Kong. About 4.3% of the antenatal population have α thalassaemia trait and 2.8% have β-thalassaemia trait (Chan et al., 1997; Sin et al., 2000). Couples with α thalassaemia or β thalassaemia trait carry 25% chance of having a fetus with either homozygous α0 thalassaemia or β thalassaemia major, respectively. Fetuses with homozygous α0 thalassaemia are not compatible with life and usually die in-utero. On the other hand, children with β-thalassaemia major require blood transfusion throughout life that carries long-term morbidity. Prenatal screening and diagnosis have been used to prevent the birth of infants with these severe forms of thalassaemia (Beris et al., 1995; Leung et al., 2004c). Prenatal diagnostic tests for thalassaemia couples include chorionic villus sampling, amniocentesis and cordocentesis to obtain samples for fetal DNA analysis (chorionic villi, amniotic fluid) or haemoglobin analysis (fetal blood). Although there is no evidence from the literature that the prevalence of common aneuploidies or other chromosomal abnormalities is higher in fetuses of thalassaemia couples, karyotyping is also performed in our practice in addition to the DNA analysis for thalassaemia.

Karyotyping or conventional cytogenetic study refers to the analysis of banded metaphase chromosomes from cultured cells. This technique identifies a wide range of chromosomal abnormalities, including alterations in copy number (aneuploidy) as well as chromosomal rearrangements, such as translocations and inversions that may be balanced or unbalanced. This technique is labour intensive. For amniotic fluid and chorionic villi cultured cells, results are not usually available in 2 weeks or more. Advances in molecular diagnostics, using either fluorescence in-situ hybridization (FISH) with specific DNA probes (Ward et al., 1993) or quantitative fluorescence–PCR (QF–PCR) with specific small tandem repeat markers (Mansfield, 1993; Pertl et al., 1994), can be applied to diagnose the common aneuploidies of chromosomes 21, 18, 13, X and Y within 1–2 days.
The sensitivity and specificity of these tests, collectively known as rapid aneuploidy diagnosis (RAD), have been demonstrated in a number of large-scale studies either using FISH (Eiben et al., 1999; Estabrooks et al., 1999; Pergament et al., 2000; Witters et al., 2002) or QF–PCR (Verma et al., 1998; Levett et al., 2001; Mann et al., 2001; Cirigliano et al., 2004). They compare favourably with karyotyping for the diagnosis of the most frequent and clinically important aneuploidies (trisomies 21, 18 and 13) as well as sex chromosome aneuploidies. These technologies will miss chromosomal abnormalities such as balanced de novo translocations and marker chromosomes (Leung et al., 2004a; Caine et al., 2005; Ogilvie et al., 2005). It has been suggested that if the indication for karyotyping is an increased risk of Down’s syndrome, such as positive screening test result or advanced maternal age, karyotyping could be effectively replaced by RAD (FISH or QF–PCR), provided there is no structural fetal abnormality detected on ultrasound examination (Thein et al., 2000; Thilaganathan et al., 2000; Ryall et al., 2001; Leung et al., 2003, Leung and Lao, 2005; Ogilvie, 2003; Ogilvie et al., 2005). The approach of using RAD as a stand-alone test instead of karyotyping when invasive prenatal testing is performed for diagnosis of thalassaemia has not been studied.

The aim of our study was to compare the detection rate of chromosomal abnormalities, when invasive prenatal testing is performed for diagnosis of thalassaemia, by four different approaches: (i) karyotyping for all samples; (ii) RAD (21,18,13,X,Y) for all samples; (iii) RAD for all samples + karyotyping for cases with ultrasound abnormalities; and (iv) RAD (21,18,13) for all + RAD (X,Y) for cases with ultrasound abnormalities consistent with Turner syndrome + karyotyping for cases with ultrasound abnormalities.

Methods

The data in this study were obtained from the database of the Prenatal Diagnostic and Counselling Department, Tsan Yuk Hospital, which is one of the referral centres for prenatal diagnosis in Hong Kong. The methods for prenatal screening and diagnosis for thalassaemia in Hong Kong has been described in a recent paper on cost-effectiveness of the programme (Leung et al., 2004c).

DNA analysis for thalassaemia was performed in the DNA diagnosis laboratory of the Department of Medicine, University of Hong Kong. Karyotyping was performed in the Prenatal Diagnostic Laboratory of Tsan Yuk Hospital.

We reviewed retrospectively the karyotype results of all prenatal samples obtained from thalassaemia couples from January 1985 to December 2002. The maternal age and the type of thalassaemia were recorded. The prevalence of common aneuploidies or other chromosomal abnormalities was compared to those published in the literature (Hook and Hamerton, 1977). The karyotype results were classified as detectable or not detectable by RAD for the common aneuploidies of chromosomes 21, 18, 13, X and Y. The presence of prenatal ultrasound fetal abnormalities and the clinical outcome of those cases with chromosomal abnormalities were retrieved from hospital records either from our obstetric unit or other referring obstetric units.

Statistical analysis was performed using the SPSS/PC 11.5 software package. The categorical variables were compared using chi-square test or Fisher’s exact test as appropriate. A P-value < 0.05 was considered statistically significant.

Results

Over a period of 18 years from January 1985 to December 2002, 1187 invasive prenatal tests were performed for the diagnosis of thalassaemia. Sixty-seven of them were excluded from the study, because of incomplete data on pregnancy outcome. The mean age of the women at sampling was 30.3 ± 4.5 years (SD) (range = 17–45 years). The type of thalassaemia couple in the studied 1120 cases included 697 α-thalassaemia couples (62.2%), 414 β-thalassaemia couples (37.0%), and 9 ωβ-thalassaemia couples (0.8%). The mean gestation when the invasive prenatal test was performed was 15.6 ± 4.0 weeks (SD) (range = 9–32 weeks). The invasive tests included 641 amniocenteses, 461 chorionic villous samplings and 18 cordocenteses.

Normal karyotypes (46,XX or 46,XY) were found in 1103 samples (98.5%). There were 17 cases (1.5%) of chromosomal abnormalities (Tables 1–3) in these 1120 samples including two cases (0.18%) of common aneuploidies (21, 18, 13), four cases (0.36%) of sex chromosome aneuploidies, one case (0.09%) of 92,XXXX, one case (0.09%) of 46,XY/46,XX, three cases (0.27%) of other aneuploidies and six cases (0.54%) of balanced translocations/inversions. The incidence of chromosomal abnormalities did not differ between α- and β-thalassaemia (P = 0.452) or between maternal age < 35 and ≥ 35 years (P = 0.214) which might be limited by the small sample size.

Table 1 summarized the four cases (0.36%) of clinically significant chromosomal abnormalities. Termination of pregnancy was chosen by the parents. Case 1 and 2 were terminated because of trisomy 21 and 18, respectively. Case 3 (45,X) was terminated because of the presence of major ultrasound abnormalities. Case 4 (47,XX,+16) had major ultrasound fetal abnormalities. This was the only clinically significant case that would have been missed if RAD had been used as a stand-alone test instead of karyotyping when invasive prenatal testing was performed for diagnosis of thalassaemia. Table 2 summarizes the eight cases (0.7%) of chromosomal abnormalities with borderline clinical significance. They included sex chromosome abnormalities (case 5 and 6), inversions (case 7, 8 and 9) and balanced translocations (case 10, 11 and 12) either inherited from one of the parents or de novo in origin. The prognosis of sex chromosome abnormalities is very different from that of trisomy 21, 18 and 13. Some obstetricians, clinical geneticists and genetic counsellors are uneasy about testing and reporting the sex chromosome status for all fetuses undergoing invasive prenatal tests. The balanced translocations and inversions were unlikely to be clinically significant for these pregnancies but may pose significant risk to future pregnancies. Table 3 summarizes the remaining five cases (0.44%) of chromosomal abnormalities that were not confirmed on subsequent prenatal (case 13, 15 and 17) or postnatal (case 14, 16) samples. They were probably a result of maternal cell contamination (case 13), confined placental mosaicism (case 14, 16 and 17) or cell culture problem (case 15). Excluding these five cases, there were five cases (0.41%) of chromosomal abnormalities: seven autosomal (case 1, 2, 4, 9, 10, 11, 12) and five sex chromosomal (case 3, 5, 6, 7 and 8) abnormalities. The overall incidence of autosomal abnormalities (7/1120 = 0.63%) was not significantly higher than that (0.41%) derived from newborn surveys for women of all ages (P = 0.398) (Hook and Hamerton, 1977). The incidence of trisomy 21, 18, 13 (case 1, 2) (2/1120 = 0.18%) was comparable to that (0.14%) from newborn surveys (P = 0.751) (Hook and Hamerton, 1977). Sex chromosomal abnormalities (5/1120 = 0.45%) were also not significantly higher than those (0.22%) from newborn surveys (P = 0.216) (Hook and Hamerton, 1977).

Discussion

In our local practice, whenever an invasive prenatal test is performed for diagnosis of homozygous thalassaemia, karyotyping is also performed to exclude the presence of chromosomal abnormalities. This strategy in prenatal diagnosis is not universal over the world. For example, in Sardinia of Italy, karyotyping is not routinely carried out for most of the cases, despite the couples’ insistence (Monni et al., 1999). This is because in their social and health system, fetal karyotyping is allowed free of charge only for women of > 35 years; in those with previous offspring affected; after the detection of ultrasound markers for chromosomal abnormalities and in cases of abnormal Down syndrome screening test results (Monni et al., 1999). In our society, this strategy would not be acceptable to the thalassaemia couples who would find an abnormal fetal karyotype unacceptable, after undergoing an invasive procedure for the diagnosis of thalassaemia. On
Clinically significant chromosomal abnormalities (case number: 1–4)

Chromosomal abnormalities with borderline clinical significance (case number: 5–12)

Chromosomal abnormalities not confirmed on subsequent prenatal or postnatal samples (case number 13–17)

1. 33 α CVS 47,XX,+21 TOP¹, placental tissue→47,XX,+21 Nil Yes
2. 47,XX,+18 TOP¹
3. 36 α Cordocentesis 45,X TOP², placental tissue→45,X Cystic hygroma, hydrops Yes
4. 47,XX,+16 TOP²; placental tissue→47,XX,+16 VSD, micrognathia, single umbilical artery No

Table I. Clinically significant chromosomal abnormalities (case number: 1–4)

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age (years)</th>
<th>Thal couple</th>
<th>Invasive test</th>
<th>Karyotype</th>
<th>Clinical outcome</th>
<th>Ultrasound abnormalities</th>
<th>Detectable by RAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>α</td>
<td>CVS</td>
<td>47,XX,+21</td>
<td>TOP¹, placental tissue→47,XX,+21</td>
<td>Nil</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>α</td>
<td>CVS</td>
<td>47,XX,+18</td>
<td>TOP¹</td>
<td>Nil</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>α</td>
<td>Cordocentesis</td>
<td>45,X</td>
<td>TOP², placental tissue→45,X</td>
<td>Cystic hygroma, hydrops</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>α</td>
<td>CVS</td>
<td>47,XX,+16</td>
<td>TOP²; placental tissue→47,XX,+16</td>
<td>VSD, micrognathia, single umbilical artery</td>
<td>No</td>
</tr>
</tbody>
</table>

CVS, chorionic villus sampling; RAD, rapid aneuploidy diagnosis; TOP¹, termination of pregnancy for chromosomal abnormalities or major ultrasound abnormalities; VSD, ventricular septal defect.

Table II. Chromosomal abnormalities with borderline clinical significance (case number: 5–12)

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age (years)</th>
<th>Thal couple</th>
<th>Invasive test</th>
<th>Karyotype</th>
<th>Clinical Outcome</th>
<th>Ultrasound abnormalities</th>
<th>Detectable by RAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>28</td>
<td>β</td>
<td>CVS</td>
<td>45,X/46,XX (74:28)</td>
<td>Livebirth, no congenital abnormality, cord blood→45,X/46,XX (5:67)</td>
<td>Nil</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>α</td>
<td>Aminio</td>
<td>47,XXX</td>
<td>TOP¹, placental tissue→47,XXX</td>
<td>Cardiomegaly, bulky placenta</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>β</td>
<td>Aminio</td>
<td>46,X,inv(Y)(p11.2;q1.23)pat</td>
<td>Livebirth, no congenital abnormality</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>β</td>
<td>CVS</td>
<td>46,X,inv(Y)(p11.2;q1.22)pat</td>
<td>Livebirth, no congenital abnormality</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>35</td>
<td>β</td>
<td>CVS</td>
<td>46,XX,inv(16)(p1q11.2)de novo</td>
<td>Livebirth, no congenital abnormality</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>α</td>
<td>Aminio</td>
<td>45,XX,rob(13:14)</td>
<td>Livebirth, no congenital abnormality</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>β</td>
<td>Aminio</td>
<td>46,XX,(t:111)(p34.3:g24)pat</td>
<td>Livebirth, no congenital abnormality</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>34</td>
<td>α</td>
<td>CVS</td>
<td>46,XY,5(p23q21.2)pat</td>
<td>Livebirth, no congenital abnormality</td>
<td>Nil</td>
<td>No</td>
</tr>
</tbody>
</table>

CVS, chorionic villus sampling; RAD, rapid aneuploidy diagnosis; TOP¹, termination of pregnancy for α- or β-thal major fetus.

Table III. Chromosomal abnormalities not confirmed on subsequent prenatal or postnatal samples (case number 13–17)

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age (years)</th>
<th>Thal couple</th>
<th>Invasive test</th>
<th>Karyotype</th>
<th>Clinical outcome</th>
<th>Ultrasound abnormalities</th>
<th>Detectable by RAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>34</td>
<td>α</td>
<td>Cordocentesis</td>
<td>46,XY/46,XX (3:2), maternal cell contamination, Aminio→46,XY</td>
<td>TOP¹</td>
<td>Hydrops</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>37</td>
<td>α</td>
<td>CVS</td>
<td>45,XY/46,XY (6:24)</td>
<td>Livebirth, no congenital abnormality, cord blood→46,XY</td>
<td>Nil, male phenotype</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>β</td>
<td>CVS, amnio</td>
<td>CVS→92,XXXX, amnio→46,XX</td>
<td>Livebirth, no congenital abnormality</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>16</td>
<td>34</td>
<td>α</td>
<td>CVS</td>
<td>47,XY,+94,XY (3:57)</td>
<td>Livebirth, no congenital abnormality</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>17</td>
<td>35</td>
<td>β</td>
<td>CVS, amnio</td>
<td>47,XY,+84,XY (8:52), amnio→46,XY</td>
<td>Livebirth, no congenital abnormality</td>
<td>Nil</td>
<td>No</td>
</tr>
</tbody>
</table>

Aminio, amniocentesis; CVS, chorionic villus sampling; NS, clinically not significant; RAD, rapid aneuploidy diagnosis by FISH or QF–PCR; thal, thalassaemia; TOP¹, termination of pregnancy for α- or β-thal major fetus; TOP², termination of pregnancy for chromosomal abnormalities or major ultrasound abnormalities; S, clinically significant; VSD, ventricular septal defect.

On the other hand, routine karyotyping (Approach 1) would imply higher and unnecessary financial costs, as most young women (< 35 years) bear babies with a normal karyotype.

We do not find a higher prevalence of chromosomal abnormalities in offspring of thalassaemia couples in our community. The incidences of both autosomal and sex chromosomal abnormalities were not higher than those from newborn surveys (Hook and Hamerton, 1977). In particular, the incidence of trisomy 21, 18, 13 (0.18%), the most common and clinically significant chromosomal abnormalities, was comparable to that (0.14%) from newborn surveys (Hook and Hamerton, 1977). One can then argue whether it is necessary to perform karyotyping for fetuses of thalassaemia couples when the risk of these common aneuploidies is no higher than that of the normal population. On the other hand, others would argue that if an invasive prenatal test has been performed, it would be unethical not to exclude chromosomal abnormalities by karyotyping.

We then studied the feasibility of using RAD as a stand-alone test (Approach 2) to replace additional karyotyping when invasive prenatal testing is performed for the diagnosis of thalassaemia. One major advantage of RAD is that the result can be available within 1–2 days as compared to 2 weeks or more for karyotyping. This becomes more important if PCR-based techniques are used in the DNA analysis for thalassaemia (Clark and Thein, 2004). As a result, the thalassaemia couple, usually very anxious, can have both the thalassaemia and RAD results within several days. In a great majority of cases (>98.5%), the couples can be reassured by the normal RAD results. The other advantage of RAD over karyotyping is that it is suggested to be more cost-effective particularly when performed on a large-scale basis (Grimsshaw et al., 2003).

The potential disadvantage of using RAD as a stand-alone test is that chromosomal abnormalities other than common aneuploidies of chromosomes 21,18,13, X and Y would be missed. It is estimated that up to four potentially clinically significant chromosomal abnormalities can be missed for every 1000 amniocenteses performed for positive Down screening test or advanced maternal age (Leung et al., 2004a,b) when RAD is used as a stand-alone
test. Our study showed that the risk of missing clinically significant chromosomal abnormalities using RAD as a stand-alone test is even less when an invasive prenatal test is performed for diagnosis of thalassaemia. This is because the prevalence of chromosomal abnormalities in offspring of thalassaemia couples is not higher than that of the normal population. Of the 12 cases (1.1%) of chromosomal abnormalities, only four of them (0.36%) were clinically significant (Table 1). Only one of these four cases was not detectable by RAD (case 4, 47,XX,+16). It is important to note that this case had major ultrasound abnormalities. If we follow the recommendation that RAD is offered to all women, restricting karyotyping to those cases with ultrasound fetal abnormalities (Approach 3) (Leung et al., 2003, 2004a,b), this single case would also be identified. For the other eight cases of chromosomal abnormalities with borderline clinical significance (Table 2), six of them were not detectable by RAD. For the five cases of chromosomal abnormalities not confirmed on subsequent prenatal or postnatal samples (Table 3), three of them were not detectable by RAD. In this regard, using RAD as a stand-alone test can have the bonus of reducing unnecessary counselling time as well as the associated iatrogenic anxiety to the couple.

Identification of sex chromosome aneuploidies, particularly in the absence of ultrasound fetal abnormalities, often poses difficult counselling issues and may not be in the best interest of the parents or the fetus, because it often presents a difficult choice regarding the continuation of the pregnancy (Donaghe et al., 2003). It is interesting to note that if the stand-alone RAD approach is further limited to chromosomes 21, 18, 13 for all cases, RAD for chromosomes X and Y only when there are ultrasound fetal abnormalities consistent with Turner syndrome (Donaghe et al., 2003), and karyotyping only performed for those cases with ultrasound fetal abnormalities (Approach 4): all four cases of clinically significant chromosomal abnormalities (Table 1) could be detected. Seven of the eight cases (except case 6 with ultrasound abnormalities) of chromosomal abnormalities with borderline clinical significance (Table 2) and four out of the five cases of chromosomal abnormalities not confirmed on subsequent prenatal or postnatal samples (except case 13 with ultrasound abnormalities) (Table 3) would not be identified unnecessarily.

Conclusion

RAD (21,18,13) for all + RAD (X,Y) for cases with ultrasound abnormalities consistent with Turner syndrome + karyotyping for cases with ultrasound abnormalities seemed to be the best approach for the detection of chromosomal abnormalities when invasive prenatal testing is performed for diagnosis of thalassaemia.

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References


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