Clinical significance of ultrasonography in screening trisomy 18 during the second and third trimesters of gestation

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Abstract: Objective To investigate the clinical significance of ultrasonography in screening trisomy 18 during the second and third trimesters. Methods Amniocentesis and cordocentesis were performed to detect karyotype of the fetus on 3128 pregnant women with indications for prenatal diagnosis during second trimester and late pregnancy. The detection rate of trisomy 18 was calculated. The relationship between the ultrasonography abnormalities and trisomy 18 was analyzed. Results In chromosomal karyotypes analysis of 3128 pregnant women by amniocentesis and cordocentesis, 14 fetus with trisomy 18 were detected, The detection rate of trisomy 18 was 0.45%. There were 211 in 3128 pregnant women with ultrasonography abnormalities, 6 fetus with trisomy 18 were found and the detection rate (2.84%) was higher than that of Down’s syndrome high risk group (0.00%), advanced age group (0.00%) and the history of abnormal deliveries group (0.00%) (all P < 0.05). No significant statistical difference of detection rate of trisomy 18 was found between ultrasound abnormality group (2.84%) and trisomy 18 high risk group (1.78%, P > 0.05). Conclusions During the second and third trimesters of gestation, ultrasonography has great significance in screening trisomy 18.

Key words: Amniocentesis; Cordocentesis; Karyotyping; Ultrasonography; trisomy; etc.

0. Introduction

It has been reported that structure abnormality of 60%-85% trisomy 18 may be detected by ultrasonography¹, Fetuses with trisomy 18 often died in intrauterine, Survivors have serious mental disorder with multiple malformations², The prognosis is poor, Most of them died within 1 years old³, so prenatal ultrasonography has great significance in screening and preventing the birth of trisomy 18. To investigate the clinical significance of ultrasonography in screening trisomy 18 during the second and third trimesters through chromosomal karyotypes analysis of 3128 pregnant women by amniocentesis and cordocentesis in this paper.

1. Clinical data and Methods

1.1 Clinical data

All subjects are pregnant women sought medical examination or consultation in Shengjing Hospital of China Medical University. Two thousand nine hundred eighty one patients with amniocentesis indications were included from January 5, 2008 - March 30, 2010, and amniotic fluid cell culture was succeeded in 2955 patients, aged 20~49 years, with a mean age of (32.4 ± 5.6) years old and 17 ~ 26 weeks of gestational age. Additional 174 patients with indications of fetal cord blood puncture were included from January 5, 2009 - March 29, 2010, and umbilical cord blood cell culture was succeeded in 173 patients, aged 19~44 years, with a mean age of (29.8 ± 4.9) years old and 24~37 weeks of gestation age. All indications of amniotic fluid puncture and umbilical cord blood sampling were detected during prenatal diagnosis, including: advanced maternal age (35 years old and above), high risk of Down syndrome (rate≥1/270), abnormal ultrasound findings, high risk of neural tube defects (NTD) and 18-trisomy syndrome, abnormal pregnancy history, history of chromosomal abnormalities of the parents, family history of genetic diseases, maternal mental retardation, drug history, viral infection and other history of obvious exposure to teratogenic factors. All pregnant women signed informed consent for post-puncture karyotyping analysis.
1.2 Methods

1) Sample collection and cell culture: ultrasound-guided amniocentesis was performed and 20~30 ml amniotic fluid was drawn for laboratory culture. Umbilical cord puncture was also performed under guidance of ultrasound, and 2 ml blood sample was placed in anticoagulant sodium heparin-treated disposable collection tube for laboratory culture.

2) Chromosome karyotype analysis: after G-band staining, cultured cells were observed and 15~30 metaphase images were counted. Three to five karyotypes were analyzed and abnormal karyotype was carefully observed and analyzed.

3) I have obtained permission from my institution’s ethics committee to perform this study and approval from my institutions review board or ethics committee.

1.3 Instruments and ultrasound methods

Voluson E8 ultrasound diagnostic apparatus by GE company was used, with probe frequency of 4~6 MHz. Through abdominal multiplanar scanning, fetal head, face, neck, chest and abdomen, internal organs, limbs and spine etc. were scanned and suspicious sites were carefully observed. During follow-up, routine measurement of relevant data was performed including fetal biparietal diameter, limb length, amniotic fluid volume and placental thickness etc. Results were recorded.

1.4 Statistical analysis

SPSS17.0 software was used for statistical analysis. Count data of each group were compared using $\chi^2$ test and $P<0.05$ was considered statistically significant.

2. Results

2.1 Chromosome karyotyping of pregnant women

In chromosomal karyotypes analysis of 3128 pregnant women by amniocentesis and cordocentesis, 14 trisomy 18 were detected with a detection rate of 0.45% (Table 1). There were 211 in 3128 pregnant women with simple ultrasonography abnormalities, and 6 of them was found with trisomy 18 with a detection rate of 2.84%; Combined with two or more puncture indications 674, 6 trisomy 18 were detected, with ultrasonography abnormalities and advanced maternal age two kind of puncture indications.

The detection rate of trisomy 18 in 3128 patients with amniotic fluid and umbilical cord blood puncture indications was shown in Table 1.

Table 1 The detection rate of trisomy 18 in 3128 patients with amniotic fluid and umbilical cord blood puncture indications

<table>
<thead>
<tr>
<th>Puncture indications</th>
<th>Patients number</th>
<th>trisomy 18 (n)</th>
<th>Trisomy 18 detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>simple ultrasonography abnormalities</td>
<td>211</td>
<td>6</td>
<td>2.84</td>
</tr>
<tr>
<td>trisomy 18 high risk</td>
<td>112</td>
<td>2</td>
<td>1.78</td>
</tr>
<tr>
<td>the Down’s syndrome high risk</td>
<td>1175</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>advanced maternal age</td>
<td>647</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>abnormal pregnancy history</td>
<td>222</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>other puncture indications</td>
<td>87</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Combined with two or more puncture indications</td>
<td>674</td>
<td>6</td>
<td>0.89</td>
</tr>
<tr>
<td>total</td>
<td>3128</td>
<td>14</td>
<td>0.45</td>
</tr>
</tbody>
</table>

$\chi^2$ test found that the detection rates of trisomy 18 among groups were significantly different ($\chi^2=53.228$ and $P<0.001$). Inter-group comparison by $\chi^2$ test found that trisomy 18 detection rate of simple ultrasonography abnormalities group (2.84%) was significantly higher than that of the Down’s syndrome high risk group (0%) , advanced maternal age group (0%), abnormal pregnancy history group (0%) (all $P<0.05$) ; No significant statistical difference of detection rate of trisomy 18 was found between ultrasound abnormality group (2.84%) and trisomy 18 high risk group (1.78%) ($\chi^2=0.042$, $P=0.837$).

2.2 Correlation of trisomy 18 with ultrasonography abnormalities
Correlation of 14 trisomy 18 with ultrasonography abnormalities and other puncture indications was shown in Table 2.

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Maternal age and gestational age</th>
<th>Abnormal karyotypes</th>
<th>ultrasonography abnormalities and other puncture indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31-year-old, 29 weeks</td>
<td>47,XN,+18</td>
<td>Fetal choroid plexus cysts</td>
</tr>
<tr>
<td>2</td>
<td>34-year-old, 35 weeks</td>
<td>47,XN,+18</td>
<td>Ventricular septal defect</td>
</tr>
<tr>
<td>3</td>
<td>22-year-old, 33 weeks</td>
<td>47,XN,+18</td>
<td>Limbs bone short; abnormal diastolic flow of umbilical artery; FGR *</td>
</tr>
<tr>
<td>4</td>
<td>31-year-old, 23 weeks</td>
<td>47,XN,+18</td>
<td>Bilateral choroid plexus cysts; bilateral lip and palate cleft; clenched hand and rocker bottom foot; ventricular septal defect; short FL and HL; FGR; polyhydramnios</td>
</tr>
<tr>
<td>5</td>
<td>25-year-old, 28 weeks</td>
<td>47,XN,+18</td>
<td>Right choroid plexus cysts; umbilical cord cyst; polyhydramnios</td>
</tr>
<tr>
<td>6</td>
<td>32-year-old, 29 weeks +1</td>
<td>47,XN,+18</td>
<td>Esophageal atresia; Polyhydramnios; single umbilical artery</td>
</tr>
<tr>
<td>7</td>
<td>42-year-old, 27 weeks +4</td>
<td>47,XN,+18</td>
<td>Single umbilical artery; short HL; High risk of 18-trisomy 1/10; advanced maternal age</td>
</tr>
<tr>
<td>8</td>
<td>40-year-old, 18 weeks</td>
<td>47,XN,+18</td>
<td>Ventricular septal defect; choroid plexus cysts; advanced maternal age</td>
</tr>
<tr>
<td>9</td>
<td>38-year-old, 21 weeks</td>
<td>47,XN,+18 (66/46, XN (34)</td>
<td>Bilateral choroid plexus cysts; short FL; advanced maternal age</td>
</tr>
<tr>
<td>10</td>
<td>28-year-old, 22 weeks</td>
<td>47,XN,+18</td>
<td>High risk of 18-trisomy 1/64; single umbilical artery; ventricular septal defect</td>
</tr>
<tr>
<td>11</td>
<td>30-year-old, 18 weeks</td>
<td>47,XN,+18</td>
<td>High risk of 18-trisomy 1/20</td>
</tr>
<tr>
<td>12</td>
<td>27-year-old, 22 weeks +2</td>
<td>47,XN,+18</td>
<td>High risk of 18-trisomy 1/270</td>
</tr>
<tr>
<td>13</td>
<td>39-year-old, 19 weeks</td>
<td>47,XN,+18</td>
<td>High risk of Down syndrome 1/44; advanced maternal age</td>
</tr>
<tr>
<td>14</td>
<td>36-year-old, 20 weeks</td>
<td>47,XN,+18</td>
<td>Abnormal pregnancy history; advanced maternal age</td>
</tr>
</tbody>
</table>

FGR * (fetal growth restriction)
Figure 1. abnormal diastolic flow of umbilical artery

Figure 2. Right choroid plexus cysts

Figure 3. Ventricular septal defect

Figure 4. three dimensional picture of Clenched hand; bilateral lip and palate cleft

Figure 1 pregnant woman was 22 years old and 33 weeks of gestation age, combined with short FL and HL; abnormal diastolic flow of umbilical artery; FGR, Ultrasonic examination showed multiple malformations of the fetus. Karyotyping of umbilical cord blood was 47, XN, +18.

Figure 2 - Figure 4 showed symptoms of the same pregnant woman, who was 31 years old and 23 weeks of gestation age, including short FL and HL; bilateral choroid plexus cysts; bilateral lip and palate cleft; clenched hand and rocker bottom foot; ventricular septal defect 0.47 cm; polyhydramnios; FGR, Ultrasonic examination showed multiple malformations of the fetus. Karyotyping of umbilical cord blood was 47, XN, +18.

3. Discussion

Chromosomal karyotypes analysis of pregnant women by amniocentesis and cordocentesis is considered the gold standard for prenatal diagnosis of trisomy 18. Invasive prenatal diagnostic techniques have certain risks, it can't be accepted by all pregnant women. Therefore, non-invasive prenatal screening should be preferentially done, karyotypes analysis is suitable for those of high risk of chromosomal abnormalities, at present, ultrasonography has been used in screening trisomy 18\(^4\).

1) Trisomy 18 syndrome is also known as Edward syndrome, is the second common trisomy, incidence is 1/4000-1/8000 of live neonatal, ratio of male/female is 1:4\(^5\), a high mortality rate, recurrence rate below 1%, is divided into four kinds of type: typical trisomy 18 (accounted for 80%, karyotype is 47, XN, +18); mosaic type (above 10%, karyotype is 46, XN /47, XN, +18), translocation and double trisomy type are rare.

Trisomy 18 syndrome has more than 130 kinds of deformity, common abnormalities including

- Cardiac abnormalities have Ventricular septal defect; Atrial septal defect; Patent ductus arteriosus (PDA); dextrocardia; larger endocardial cushion defects; transposition of the great arteries; Common atrioventricular canal defects; double outlet right ventricle; aortic or pulmonary stenosis; coronary artery malformation;
- Abnormalities of limbs and skeletal system finger buckling; overlapping finger is one of the most obvious and characteristic abnormalities of trisomy 18 syndrome, other malformations have syndactyly; polycystic; rocker bottom foot; foot varus and valgus; many finger arch pattern; pass through palm; short limb deformity; radial dysplasia or lack; thumb dysplasia or lack;
- Fetal growth restriction (FGR);
- Facial deformity have micrognathia; microtia; low-set ears; outer ear malformation; small mouth; wide eye distance; small eyes; small palpebral fissure; lip and palate cleft;

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The central nervous system abnormalities have hydrocephalus; ventriculomegaly; choroid plexus cysts; agenesis of the corpus callosum; meningoceles or meningomyelocele; microcephaly; Dandy-Walker deformity; cisterna magna broadening; small cerebellar; strawberry head—one of the most important features of trisomy 18; Fetal genitourinary tract abnormalities have multicystic dysplastic kidney; horseshoe kidney; hydrorephrosis; amniotic fluid; Abnormalities of the fetal gastrointestinal tract have Esophageal atresia; tracheoesophageal fistula; anal rectum atresia; omphalocele; diaphragmatic hernia; Adjunct anomaly have polyhydramnios or oligohydramnios; single umbilical artery; Umbilical cord blood flow resistance index on the high side; small placenta; battledore placenta; umbilical cord cyst; Umbilical vein tumor;
2) Occurrence mechanism of trisomy 18 It is mainly that chromosomes of oocyte are not separated during meiosis, most is in mitosis II period, Maternal meiosis chromosome non-disjunction accounted for 90%, father body meiosis chromosome non-disjunction accounted for 5%, The others is possibly relevant to parents chromosomes balanced translocation transfer. advanced maternal age is main reason of occurrence of trisomy 18, In this study, advanced maternal age accounted for 35.71% (5/14), non-advanced maternal age accounted for 64.29% (9/14), so prenatal screening of pregnant women under 35 should be paid more attention.
3) The clinical significance of ultrasonography in screening trisomy 18 In this study, as shown in Table 1-2, 14 fetuses with trisomy 18, ultrasound screening detected 6 cases of trisomy 18, while ultrasound screening combined with other puncture indications detected 4 cases of trisomy 18, with a total trisomy 18 detection rate of 71.43% (10/14), is similar to 77% of the report of Jae etc. [6]. The detection rate by ultrasonography abnormalities was higher than that of Down's syndrome high risk group, advanced age group and the history of abnormal deliveries group. This suggests that ultrasound is an effective screening method for trisomy 18.

main ultrasonography abnormalities in 14 fetuses with trisomy 18 including Choroid plexus cysts There are 5 fetuses with choroid plexus cysts, accounted for 35.71% (5/14), is similar to 77% of the report of Brumfield etc. [7]. 4 fetuses Combined with other ultrasonography abnormalities (Figure 2- Figure 4) , 1 fetus is along with choroid plexus cysts, Beke etc. [8] studied the risk of chromosomal abnormalities of choroid plexus cysts, find whether unilateral or bilateral, with or without other ultrasonography abnormalities, the risks of chromosomal abnormalities are all increasing. A single choroid plexus cysts, the risk of fetus with trisomy 18 is 1.5 times higher than that of background [9].
Cardiac abnormalities Yang etc. [10] think that cardiac abnormalities are the most common to fetuses with trisomy 18 after 16 weeks. Reports suggested that there is single cardiac abnormalities in trisomy 18 [11]. Other reports suggested that 16% of fetuses with normal cardiac structure alone had chromosome abnormalities, and 66% fetuses combined with other structural abnormalities had chromosomal abnormalities [12]. In this study, 4 cases of ventricular septal defects were detected among trisomy 18 fetuses (1 case of simple ventricular septal defect and 3 cases of ventricular septal defect combined with multiple fetal malformations) (Figure 2- Figure 4), accounting for 28.14% (4/14);
Umbilical cord abnormalities There are 5 fetuses with umbilical cord abnormalities in this study, accounting for 35.7% (5/14); have single umbilical artery (3/14); umbilical cord cyst (1/14); abnormal diastolic flow of umbilical artery (1/14) (Figure 1), All fetuses with umbilical cord abnormalities are combined with other ultrasonography abnormalities;
Polyhydramnios 3 pregnant women with trisomy 18 fetuses in our study had polyhydramnios, accounted for 21.43% (3/14);
FGR FGR is common clinical manifestations of trisomy 18 fetuses. There are 2 fetuses with FGR in this study, accounted for 14.29% (2/14), Nyberg etc. [13] reported that a group of trisomy 18 fetuses less than 24 weeks of gestational age, 28% with FGR, more than 24 weeks of gestational age, 68% with FGR.
Abnormalities of limbs and skeletal system Viora etc. [14] report that Abnormalities of hand and foot (40.8%) and FGR (35.2%) are the most common Abnormalities of trisomy 18 fetuses, There are 5 fetuses with Abnormalities of limbs and skeletal system in this study, accounting for 35.71% (5/14), including short limbs (2/14), short FL, short HL and Clenched hand respectively (1/14) (Figure 4).

Most of the trisomy fetuses have 2 or more abnormal ultrasound findings, so detection of more than 2 ultrasonic signs suggests possible aneuploidy [15]. In this study, more than 2 ultrasonic signs were identified in 8 fetuses.
4) False-negative rate of ultrasound screening as shown in Table 2, 4 fetuses with normal ultrasound evidences were confirmed as trisomy 18 through other further puncture detections, accounting for 28.57% (4/14) of the trisomy 18 detection rate, suggesting that the normal ultrasound results in these patients are false negative. If we have not performed karyotype analysis based on maternal age, serum screening and other adverse reproductive history of the pregnant women, we would have missed the diagnosis of trisomy 18 in these patients. Therefore, joint screening methods can reduce the false negative rate of ultrasound screening and improve the detection rate of trisomy 18. Ultrasonography during the first and second trimesters combined with serum AFP, β-HCG, UE3 of pregnant women may increase the detection rate of trisomy 18[16].

4. Conclusion

In summary, ultrasonography in screening trisomy 18 has great significance during the second and third trimesters.

REFERENCES