Clinical significance of ultrasonic detection of fetal nuchal fold thickening in screening trisomy 21 during the second trimester

PAN Yu-ping1, 2

1. Medical College, Eastern Liaoning University, Dandong, Liaoning118003 China;
2. Department of Ultrasound, Shengjing Hospital of China Medical University, Shenyang, Liaoning110004 China
E-mail: Panxy900@sina.com

Abstract: Objective To investigate the significance of ultrasonic detection of fetal nuchal fold (NF) thickening in screening trisomy 21 during the second trimester. Methods Amniocentesis and cordocentesis were performed in pregnant women with indications of prenatal diagnosis, and the chromosome karyotype analysis was performed. The detection rate of trisomy 21 in fetus with ultrasonic manifestation of NF thickening was calculated. Meanwhile, the relationship between fetal NF thickening and the occurrence of trisomy 21 was observed. Results The chromosome karyotype analysis of the pregnant women who underwent amniocentesis showed that there were 18 fetuses with NF thickening. Trisomy 21 was found in 5 fetuses among them, the detection rate was 27.78%. The detection rate of trisomy 21 detected hinted by fetal NF thickening was higher than that of other abnormal ultrasonic manifestations (P = 0.015). The chromosome karyotype analysis of the pregnant women who underwent cordocentesis showed that there were 12 fetuses with NF thickening, among which 1 of trisomy 21 was detected, the detection rate of trisomy 21 was 8.33%. Conclusions Fetal NF thickening is an effective soft marker of ultrasonography in screening trisomy 21 during the second trimester.

Key words: Ultrasonography, prenatal; Fetus; Amniocentesis; Down syndrome; Cordocentesis; Karyotyping; etc.

0. Introduction

Ultrasonic testing NF has certain sensitivity and specificity to identify trisomy 21. This paper investigate the clinical significance of ultrasonic detection of fetal nuchal fold (NF) thickening in screening trisomy 21 during the second trimester through chromosomal karyotypes analysis of 2955 pregnant women amniotic fluid cell culture and 173 pregnant women umbilical cord blood cell culture.

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 29, 2010, 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, 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 institution’s 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.

NF thickening is measured in the cerebellum cross section, image magnification to fetal head more than half of the screen, specific measurement: scanning fetal head cross-section, after showing transparent every and thalamus, display clearly cerebellum probe backwards into angle, measure the distance between skull pillow department lateral margin and the skin lateral margin in the central level, Namely NF, take an average by measuring 2 times, NF thickening (16 ~ 18 weeks of gestational age ) ≥5 mm and NF thickening (19~ 24 weeks of gestational age ) ≥6 mm is the standard of judging NF thickening. Cut should avoid excessive tilt to below after pillow, lest make the measured values too large. Gestational age, fetal position and whether umbilical cord is around the neck etc will influence measurement of NF thickness. NF thickening should distinguish with water bag tumor of fetal back and neck.

To acquire nuchal fold thickness index (nuchal index, NIX), calculation formula: NIX = NF thickness (mm)/biparietal diameter (BPD)(mm)×100%.

1.4 Statistical analysis

SPSS13.0 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

1) Cell culture. 2981 pregnant women received amniocentesis, and amniotic fluid cell culture was succeeded in 2955 patients, with a success rate of 99.13%. 174 pregnant women received cord blood puncture, and umbilical cord blood culture was succeeded in 173 patients, with a success rate of 99.43%.

2) Chromosome karyotyping of pregnant women: amniotic fluid cell karyotyping was analyzed in 2955 patients, and abnormal karyotype was detected in 150 patients, with a detection rate of 5.08%. In chromosomal karyotypes analysis of 2955 pregnant women with amniocentesis, 37 trisomy 21 syndrome were detected. By prenatal ultrasound examination, there were 18 fetus with NF thickening. 5 trisomy 21 syndrome were detected, with a detection rate of 27.78%. 74 found other ultrasound abnormalities, 4 trisomy 21 syndrome were detected, with a detection rate of 5.41%. The detection rate of trisomy 21 detected hinted by fetal NF thickening was higher than that of other abnormal ultrasonic manifestations ($P=0.015$). Among 37 trisomy 21 syndrome detected by amniotic fluid cell culture, 5 ultrasonic manifestation of NF thickening, accounting for 13.51% of trisomy 21. Among normal karyotype fetus, there were 12 fetus with NF thickening (19~26 weeks of gestational age), accounting for 0.43%.

3) Umbilical cord blood puncture karyotyping was analyzed in 173 patients, and abnormal karyotype was detected in 26 patients, with a detection rate of 15.03%. In chromosomal karyotypes analysis of 173 pregnant women with umbilical cord blood cell culture, 8 trisomy 21 syndrome were detected, there were 12 fetus with NF thickening by prenatal ultrasound examination, among them 1 trisomy 21 syndrome were detected (as is shown in figure 1), with a detection rate of 8.33%. 106 found other ultrasound abnormalities, 5 trisomy 21 syndrome were detected, with a detection rate of 4.72%. Difference of the detection rate of trisomy 21 detected hinted by fetal NF thickening and other abnormal ultrasonic manifestations was no statistical significance ($P=1.000$). Umbilical cord blood cell culture, 8 trisomy 21 syndrome were detected, there were 1 fetus with NF
thickening, accounting for 12.5% of 21 trisomy. Among normal karyotype fetus, there were 11 fetus with NF thickening (25 ~ 30 weeks of gestational age), accounting for 7.48%.

4) Related material of trisomy 21 syndrome with NF thickening was shown in Table 1.

### Table 1  Related material of trisomy 21 syndrome with NF thickening

<table>
<thead>
<tr>
<th></th>
<th>BPD(cm)</th>
<th>Abnormal karyotypes</th>
<th>NF thickness (cm)</th>
<th>Nix</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 years old, 24 weeks</td>
<td>6.0</td>
<td>47, XN, + 21 (amniotic fluid)</td>
<td>0.62</td>
<td>10.33</td>
</tr>
<tr>
<td>37 years old, 22^1 weeks</td>
<td>4.6</td>
<td>47, XN, + 21 (amniotic fluid)</td>
<td>0.62</td>
<td>13.48</td>
</tr>
<tr>
<td>28 years old, 24^2 weeks</td>
<td>5.1</td>
<td>47, XN, + 21 (amniotic fluid)</td>
<td>0.73</td>
<td>14.31</td>
</tr>
<tr>
<td>31 years old, 22^1 weeks</td>
<td>5.6</td>
<td>47, XN, + 21 (amniotic fluid)</td>
<td>0.81</td>
<td>14.46</td>
</tr>
<tr>
<td>36 years old, 23^3 weeks</td>
<td>5.8</td>
<td>47, XN, + 21 (amniotic fluid)</td>
<td>0.61</td>
<td>10.52</td>
</tr>
<tr>
<td>11 years old, 23^n weeks</td>
<td>5.7</td>
<td>47, XN, + 21 (umbilical cord blood)</td>
<td>0.77</td>
<td>13.51</td>
</tr>
</tbody>
</table>

Related material of trisomy 21 syndrome with other ultrasound abnormalities was shown in Table 2.

### Table 2  Related material of trisomy 21 syndrome with other ultrasound abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Abnormal karyotypes</th>
<th>Abnormal ultrasonic manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>46 years old, 22 weeks</td>
<td>47, XN, + 21 (amniotic fluid)</td>
<td>The left lateral ventricle is about 1.2 cm wide</td>
</tr>
<tr>
<td>25 years old, 22^1 weeks</td>
<td>47, XN, + 21 (amniotic fluid)</td>
<td>The left choroid plexus cyst, femoral short</td>
</tr>
<tr>
<td>30 years old, 28 weeks</td>
<td>47, XN, + 21 (umbilical cord blood)</td>
<td>Bilateral lateral ventricle is about 1.1 cm wide</td>
</tr>
<tr>
<td>33 years old, 25 weeks</td>
<td>47, XN, + 21 (umbilical cord blood)</td>
<td>Edema, fetal chest and abdominal cavity effusion, femoral short</td>
</tr>
<tr>
<td>40 years old, 28 weeks</td>
<td>47, XN, + 21 (umbilical cord blood)</td>
<td>Fetal bowel expansion, polyhydramnios</td>
</tr>
<tr>
<td>26 years old, 33 weeks</td>
<td>47, XN, + 21 (umbilical cord blood)</td>
<td>Fetal duodenal atresia, limbs short</td>
</tr>
<tr>
<td>31 years old, 35 weeks</td>
<td>47, XN, + 21 (umbilical cord blood)</td>
<td>Fetal chest and abdominal cavity effusion, polyhydramnios, limbs short</td>
</tr>
<tr>
<td>38 years old, 18 weeks</td>
<td>47, XN, + 21 (amniotic fluid)</td>
<td>femoral short</td>
</tr>
</tbody>
</table>
Figure 1 pregnant women, 40 years old, 23+6 weeks of gestational age, karyotype is 47,XN,+21 (umbilical cord blood).

Ultrasonography hinted: fetal NF thickness is 0.77 cm, fetal limbs short.

3. Discussion

Major methods for prenatal diagnosis of trisomy 21 include serum screening, ultrasonography and karyotype analysis, the first two as non-invasive examinations[2] and the last one as invasive examination, which is considered the gold standard for prenatal diagnosis of trisomy 21.

1) Trisomy 21 syndrome also called Down's syndrome, is the most common chromosomal abnormality, Neonatal incidence is about 1/500-1/700, common in older woman with mental retardation and multiple deformity as main characteristics, and have special face, trisomy 21 the main structure deformity and tiny lesions have: ① Neck transparent layer (NT) or NF thickening; ② Brain abnormality (mild ventricle expansion, choroidal plexus cyst, cerebellum little volume); ③ Facial abnormality (wide distance between the eyes, nose bone dysplasia or absent, nasal root low etc); ④ Cardiac anomalies (atrial septal defect, ventricular septal defect, common atrioventricular canal defects etc); ⑤ Abdomen abnormal (duodenal intestinal atresia, intestinal strong echoes, acromphalus); ⑥ Limbs abnormal (femur or humerus short, the little finger section in finger bone dysplasia, up the palm); ⑦ Iliac Angle increases; ⑧ Mild renal pelvis expansion; ⑨ Fetal edema, the chest abdominal cavity effusion, and polyhydramnios; ⑩ Intrauterine growth retardation (IUGR); the renal pelvis expansion, cardiac anomalies, choroid plexus cyst, femur or humerus short, duodenal atresia, ventricle expansion etc. among them trisomy 21 syndrome the value of screening existing literature[3-5] reported.

2) The formation mechanism of NF thickening. The pathophysiological basis of NF thickening is not completely understood, may be relevant with the following factors[6-7]: ① Early pregnancy lymphatic water sac tumor evolved; ② Various reasons cause lymphatic system backflow impeded, vein obstruction to flow, vein congestion; ③ Fetal heart function failure; ④ Chromosom abnormality; ⑤ Quickened disappear; ⑥ Accepting blood fetus of twin-twin transfusion syndrome (TTTs); ⑦ Alpha Mediterranean anemia homozygote fetuses.

3) The relationship between fetal NF thickening and trisomy 21. NF thickness is one of the trisomy 21 syndrome main signs. benacerraff etc reported for the first time ultrasonic detection of NF thickening increase the risk of with Down syndrome during the second trimester in 1985. Studies show[6-9] NF thickness ≥6 mm, compared with the normal fetus, the risk of trisomy 21 increase 17 times. Literature[10] reported 2.6%-65% of trisomy 21 can have NF thickening, and normal fetus about 0.1% can have NF thickening, in addition, fetus of trisomy 13, trisomy 18 and 45, X abnormal karyotype can also have NF thickening.

Some scholars[11] define NF as fetal nuchal fold thickening skin edema after neck leading to 15 ~ 20 weeks of gestational age. In the fetus of NF thickness ≥6mm 24 weeks or so of gestational age this group found 4
trisomy 21 (Table 1), accounts for 66.67% (4/6) of trisomy 21 detected by prenatal ultrasonography, so the author think that it may be more reasonable to test NF at 15 ~ 24 weeks.

In this study, amniotic fluid puncture karyotype analysis, the fetus of prenatal ultrasound shows NF thickening detected 5 trisomy 21. The detection rate of trisomy 21 detected hinted by fetal NF thickening was higher than that of other abnormal ultrasonic manifestations, hinted that testing NF at 15 ~ 24 weeks is helpful to improve the detection rate of trisomy 21. In this group, the fetal umbilical cord blood puncture karyotype analysis, the fetus of prenatal ultrasound shows NF thickening detected 1 trisomy 21, detected 5 trisomy 21 by finding other abnormal ultrasonic manifestations. Difference of the detection rate of trisomy 21 detected hinted by fetal NF thickening and other abnormal ultrasonic manifestations was no statistical significance, the reasons may be by analyzing ① the most (11/12) fetus of prenatal ultrasound shows NF thickening is more than 24 weeks (25 ~ 33 weeks of gestational age), testing NF thickness have little significance to the detection of trisomy 21 in this time; ② the fetal gestational age of found other abnormal ultrasonic manifestations is 25 ~ 33 weeks, if found fetal limbs short, polyhydramnios, lateral ventricle expansion, duodenal atresia, bowel expansion, chest and abdominal cavity effusion, fetal entire edema, choroid plexus cyst, renal pelvis expansion etc ultrasound soft marker[12] have larger significance to the diagnosis of trisomy 21 in this time, the literature[13] reports, when ≥2 abnormal ultrasonic manifestations exist, the sensitivity and specificity of diagnosing trisomy 21 were 68% and 98% respectively. In this study, 9 trisomy 21 detected by other abnormal ultrasonic manifestations, 6 trisomy 21 abnormal ultrasonic manifestations ≥2, accounts for 66.67%. Nix measured values do not affect gestational age, the literature[14] reports, when Nix>11 the sensitivity and specificity of diagnosing fetal chromosomal abnormalities were 50% and 96% respectively, 6 trisomy 21 of NF thickening in this group, 4 trisomy 21 Nix>11, accounts for 66.67%.

4. Conclusion

In short, fetal NF thickening is an effective soft marker of ultrasonography in screening trisomy 21 during the second trimester.

REFERENCES