Prenatal screening and prenatal diagnosis with cordocentesis for prenatal chromosome abnormalities

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Abstract

Objective: various prenatal diagnosis examinations greatly contribute to predicting chromosome abnormalities, cordocentesis is one of the important methods for prenatal diagnosis in China. Materials and methods: Pregnant women underwent serum screening and sonographic screening, who were conducted on umbilical cord blood because of high-risk pregnancy abnormalities, the umbilical cord blood was brought up to culture medium, lymphocytes were collected for karyotype analysis. Results: we found that a total of 112 (4.8%) cases were diagnosed with chromosomal abnormalities, including 72 cases (64.3%) of trisomy syndrome, 15 cases (13.4%) of sex chromosome aneuploidy, 12 cases (10.7%) of translocation, 7 cases (6.3%) of mosaic and 6 other types (5.4%). In the serum screening abnormalities, compared to the abnormalities of multi-markers and single markers, the multi-markers showed statistically significant difference in chromosome abnor malities. In the abnormal group of ultrasound screening, the structural anomalies showed statistically significant difference in chromosome abnormalities compared to anomalies of sonographic marker (14.8%vs.3.5%). Patients without abnormalities were found in groups with rare characteristics. the high-risk compound and abnormalities of single and multiple serum markers were important in predicting chromosomal disorders. Compared with sonographic markers, fetal structural abnormalities were more effective in predicting chromosomal abnormalities. Conclusion: cordocentesis is crucial significance for the diagnosis of fetal chromosome abnormalities, especially for those gravidas with advanced maternal age who have not received regular prenatal examinations.

Keywords

Cordocentesis;Prenatal Diagnosis; Serum Screening; Ultrasound Screening.

1. Introduction

Since cordocentesis was first introduced in 1983, it has been performed as a reliable prenatal diagnostic method. Within 2 weeks after cordocentesis, the rate of cordocentesis-related fetal loss was 1.0%[1]. but the rates of 16 and 24 weeks' gestation with obvious fetal anomaly were 3.2% and 1.8%[2], respectively. fetal loss had a significantly higher rate in placenta penetration, low birth weight and preterm birth and so on[3]. With these disadvantages, when chorionic villus sampling or amniotic fluid culture are impossible, a quick karyotyping is requested, and cordocentesis is still a useful method for early diagnosis.

Prenatal screening and genetic diagnosis are important in obstetrics. Noninvasive procedures, maternal serum analysis and ultrasound screening for chromosomal disorders are now routinely offered for women during pregnancy[4-7]. Invasive procedures, chorionic villus sampling, amniocentesis and cordocentesis are used as prenatal diagnosis methods. For screening 21-trisomy, 18-trisomy and other chromosome abnormalities, in the first trimester[8]. pregnancy-associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin (β-hCG) and nuchal translucency (NT) are recommended to be detected; in the second trimester, maternal serum screening markers, alpha-fetoprotein (AFP), β-human chorionic gonadotropin (β-hCG), unconjugated estriol (uE3), inhibin A and ultrasound screening are recommended. In Turkey, triple test screening is routinely offered to all gravidas[9]. Ultrasound screening plays an important role in detecting congenital anomalies in the second trimester, and it is a routine method of antenatal care in the industrialized world[7, 10]. Ultrasound screening is the most sensitive test for diagnosis of trisomy 18[11, 12]. however, most screening results with abnormality were needed to further diagnosis with invasive procedure of fetal karyotyping[13].

Here, a retrospective study of 2342 cordocentesis cases was conducted to investigate the predictors of prenatal chromosome abnormalities over a 13-year period. we summarized the result of conventional screening indicators and focused on the cytogenetic results of cordocentesis. Although studies had reported various indications for cytogenetic analysis, which are rare reported in China, especially in cordocentesis. Thus, this article is mainly focused on cordocentesis in China.

2. Materials and methods

2.1 Subjects

This retrospective study was conducted on umbilical cord blood of 2354 cases who had high-risk pregnancy abnormalities, 12 cases were not obtained for severe thalassemia or fetal death. This study was undertaken with the approval of the Ethics Committee of Shenzhen people's hospital Human Subjects Review Committee, Guangdong, China. Written informed consent was obtained from all participated. before surgery, the complications and risks of cordocentesis were explained in detail to the patients, who signed the informed consent for cordocentesis. Maternal ages were from 19 to 46 years old with a $\bar{x}\pm s$ of 29.3±4.7 years.

2.2 Cordocentesis Procedures

Using a real-time ultrasonographic scanner to locate the placenta and choose a puncture site that was easily visualized, cordocentesis was performed using the freehand technique and transabdominal insertion as previously described[14]. Briefly, the placenta and insertion site were located by ultrasonographic scanner, and then a regular 22-gauge spinal needle was used, 0.5-2 mL umbilical cord blood was used for karyotyping, while hemoglobin electrophoresis was performed to determine whether the blood was from the fetus.

2.3 Cell Culture

The umbilical cord blood was brought up to culture medium, 37°C, 5% CO_2.

2.4 G-banding Karyotype Analysis

After 72h, colcemid was added, and lymphocytes were collected for karyotype analysis after 3h. G banding was performed by the International System for Human Cytogenetic Nomenclature 2013(ISCN 2013) standard.

2.5 Serum Screening

First and second maternal serum screening markers for chromosome abnormalities include PAPP-A, alpha-fetoprotein (AFP), β-human chorionic gonado tropin (β-hCG) and unconjugated estriol (uE3). Concentrations of the above analytes were expressed as multiples of the median (MoM) for unaffected pregnancies. Compound high risk for common trisomy syndromes was defined as T21≥1/270 and T18≥1/350. Individual abnormal markers with compound low risk refer to maternal serum concentrations at or below specified cut-offs: β-hCG≤0, MoM or PAPP-A≤0.43, MoM or

AFP≤0.6, MoM or uE3≤0.73, MoM or AFP+Age≥ 1/270; multiple abnormal serum markers were defined as two or more individual abnormal markers with compound low risk.

2.6 Sonographic Screening

Sonographic screening was considered abnormal when at least one anomaly was found. According to reports by Estroff *et al*[15]and Filkins *et al*[16], major or structural anomalies include cardiac defects, duodenal atresia, ventriculomegaly, spina bifida, etc.; sonographic markers include absent nasal bone, nuchal thickening, echogenic bowel, echogenic intracardiac focus (EIF), choroid plexus cysts, rocker bottom feet, polyhydramnios, IUGR, （intrauterine growth retardation infant, IUGR) shortened proximal long bones (humerus and femur), pyelectasis, single umbilical artery, pericardial effusion, and R/L （right/lift ）heart disproportion. Other characteristics were defined as rare abnormalities, including various system tumors or other undetermined sonographic abnormalities.

2.7 Statistical Analysis

All the distributions of maternal age, gestational age and obstetric history were skewed, so a Wilcoxon rank sum test of two independent samples was used for statistical analysis between the abnormal and normal group. Chi-square test was used for statistical analysis of differences in fetal sex and detection rate of different groups of each cordocentesis. A two-tailed *P* value of less than 0.05 was considered statistically significant. All of the statistical analyses were performed using SPSS version 17.0.

3. Results

a total of 2354 gravidas underwent cordocentesis. Ultimately, 2342 cases were involved in the study because others failed severe thalassemia or fetal death. The successful rate of cord blood culture was 99.5%. According to cytogenetic evaluation, a total of 112 patients were diagnosed with chromosomal abnormalities, including 72 cases (64.3%) of trisomy syndrome (40 21-trisomy, 18-trisomy, 11 13-trisomy), 15 cases (13.4%) of sex chromosome aneuploidy (5 Turner syndrome, 5 cases 47, XYY syndrome, 3 trisomy X, 2 Klinefelter syndrome), 12 cases (10.7%) of translocation (6 Robertsonian translocation, 6 other autosome translocation), 7 cases (6.3%) of chromosome mosaic and 6 other types (5.4%) that included abnormalities of marker chromosome quantity and structure (table 1). In the abnormal group, the mean maternal age was 31.3±5.6 years, significantly older than the normal karyotype group(29.2±4.6 years). There were no statistically significant differences in terms of punctual gestational age, obstetric history or fetal sex between abnormal and normal karyotypes (table 2). Chromosomal variants are considered to be normal, such as enlarged heterochromatin and satellites. According to ISCN (2009) standards for karyotype analysis, 46, XY(XX), inv(9)(p12q13) and inv(Y)(p11.2q11.2)were also not considered chromosome abnormalities.

In the present study, many patients had two or more indications for cordocentesis, which were analyzed separately for statistical analysis. Here, we focus on usual indications in Southern China, including advanced maternal age, abnormal serum screening, ultrasound findings, history of intrauterine fetal death or abortion, fetal risk for thalassemia and positive result of TORCH test.

Table 1 Abnormal karyotype, numbers and proportion

Abnormal karyotype	numbers	Proprotion (%)
Trisomy	72	64.3
Sex chromosomal aneuploidy	15	13.4
Translocation	12	10.7
Mosaic karyotype	7	6.3
Others	6	5.4
Total	112	100

Table 2 Comparison between fetus with abnormal and normal karyotypes

	Abnormal group (n=112)	Normal group (n=2230)	P value
Maternal age	31.3±5.6	29.2±4.6	<0.001
Punctual gestational weeks	24.4±3.5	24.4±3.3	0.977
Gravidity (n)	2.3±1.4	2.3±1.3	0.925
Parity (n)	0.4±0.6	0.4±0.6	0.921
Fetal sex ratio (M:F)	59/53 (1.1)	1204/1026 (1.2)	0.786

Fetal sex: Pearson Chi-square χ^2=0.074, df=1; M:F means male: female.

Underwent cordocentesis, 31 abnormal fetus were found in 364 cases with advanced ages (≥35 years). 81 abnormal fetus were found under 35 years old. Chi-square test showed that the difference was statistically significant in the abnormal karyotypes between the two groups (table 3).

Table 3 Comparison of abnormal karyotypes between gravidas <35 years and those ≥35 years

Group	abnormal karyotypes (n)	Percentage	Total (n)
<35 years	81	4.1%	1978
≥35 years	31	8.5%	364
Total	112	4.8%	2342

Serum screening and abnormal karyotypes are presented in table 4. In abnormal serum screening results, 81 fetus had abnormal chromosomes (6.1%), and 31 fetus of abnormal chromosomes were found in normal serum screening results (3.0%). Chi-square test showed statistically significant difference in detection rate of chromosome abnormalities between the two groups. and then we clarified the relationship between serum results and chromosome abnormalities, divided the abnormal serum screenings into 3 sub-groups: compound high-risk group, single abnormal marker with compound low-risk group, and multiple abnormal markers with compound low-risk group. Percentage of chromosome abnormalities was 5.0%, 5.6%, and 15.7%(Table 4), respectively. Compared to the compound high-risk group and single abnormal marker group, the group with multiple abnormal serum markers was statistically significant difference. There was no significant difference between the compound high-risk group and single abnormal marker group.

Table 4 Serum screening results

Groups	chromosome abnormalities (n)	Total (n)	Percentage (%)
Group with abnormal serum screening results			
Compound high-risk group	30	596	5.0
Single abnormal marker with low compound risk	35	623	5.6
Multiple abnormal markers with low compound risk	16	102	15.7**
Total	81	1321	6.1*
Group with normal serum screening results	31	1021	3.0

*: *P*=0.000 *vs.* group with normal serum screening results (χ^2=12.118); **: *P*=0.000 *vs*. compound high-risk group (χ^2=16.055), *P*=0.000 *vs*. single abnormal marker with low compound risk (χ^2=13.587).

In the groups with abnormal and normal ultrasound screening results, 55 and 57 fetus were identified chromosome abnormalities, respectively (table 5). Sonographic screening plays crucial rule in the trimester of prenatal screening, we categorized the group of abnormal ultrasound screenings into 3 sub-groups, including structural anomalies, sonographic anomalies and others.

In the structural anomalies and sonographic anomalies, 14.8% and 3.5% chromosome abnormalities were discovered, respectively. Chi-square test showed statistically significant difference in the rate of abnormal karyotypes.

Table 5 Ultrasound screenings

Groups	chromosome abnormalities (n)	Total (n)	Percentage (%)
Abnormal ultrasound screenings			
Structural anomalies	48	324	14.8**
Sonographic anomalies	7	198	3.5
Others	0	7	0
Total	55	529	10.4*
Normal ultrasound screening results	57	1813	3.1
Total	112	2342	4.8

*: P=0.000 *vs.* the normal ultrasound (χ^2=47.310); **: P=0.000 *vs*. the sonographic anomalies

History of intrauterine fetal death or abortion was also an indication for cordocentesis. 115 cases had history of fetal death or abortion in this study. Three abnormal karyotypes were found among them, including 47, XYY, trisomy 21 and trisomy 13, which cases had other indications concurrently. whereas other 2227 gravidas without this indication, 109 abnormal karyotypes were identified.

Other indications included radiation, genetic disease, balanced translocation of chromosome, intermarriage, albinism or deafness gene detection, anxiety and so on in the trimester, 4 abnormal karyotypes (3.2%) were found in 125 cases with other indications, including 46, XX[40]/45, X0[10], trisomy X, 45,XX, rob(13;15) (q10; q10)mat and 47, XX, +der(22?). In 2217 cases without history of intrauterine fetal death or abortion, 108 abnormal karyotypes (4.9%) were identified. There was no significant difference in percentage of abnormal chromosomes between the two groups.

Fetus with thalassemia and positive TORCH results were also common indicators for cordocentesis in southern China. 414 fetus with high risk of thalassemia, diagnosed 263 α-thalassemia, 93 β-thalassemia, and 4 abnormal hemoglobinopathy. The remaining were normal. 10 chromosome abnormalities with thalassemia were identified, including 46, XY, del (7) (q34:) and others, (46, XY, del (7) (q34:)) was discovered by high risk for thalassemia. In the 411 cases with high risk thalassemia, 40.1% pregnant women had abnormal serum results at the same time. which 165 cases with positive TORCH results underwent cordocentesis, 3 fetus were found chromosome abnormalities, 1 case of trisomy 21 was discovered by positive TORCH results only, and others had other indications at the same time.

Table 6 shows the distribution of various chromosomal abnormalities, 112 chromosome abnormalities were discovered by cordocentesis in our center. Among them, 72 fetus with trisomy syndrome were detected, including 40 21-trisomy, 21 18-trisomy and 11 13-trisomy. only 85.0% 21-trisomy, 85.7% 18-trisomy and 72.7% 13-trisomy could be detected by serum screening. Among the 34 21-trisomy cases with abnormal serum screening results, 22 showed high-risk compound for aneuploidy, 9 cases had single abnormal marker, and 3 had multiple abnormal markers. 10 cases of 18-trisomy had single abnormal serum marker in abnormal serum screening results, 8 remaining

cases had multiple abnormal markers. Using ultrasound screening, 25.0% 21-trisomy, 85.7% 18-trisomy and 90.9% 13-trisomy could be detected only. In addition, sonographic screening is superior to other indications in fetus with abnormal chromosomes (Table 6). 40.0% 21-trisomy, 14.3% 18-trisomy and 9.1% 13-trisomy could be detected only by advanced maternal age. Combining serum markers with ultrasound screening, 92.5% 21-trisomy, 95.2% 18-trisomy, 100% 13-trisomy and 89.3% other chromosome abnormalities were identified. Adding maternal age, 97.5% 21-trisomy, 100% 18-trisomy, 100% 13-trisomy and 95.5% other chromosome abnormalities could be detected prenatally.

Table 6 Distributions of main indications for various chromosome abnormalities

	T21	T18	T13	SCA	Translocation	Mosaic	Others	Total
Karyotypes	40	21	11	15	12	7	6	112
Percentage	100%	100%	100%	100%	100%	100%	100%	100%
Advanced maternal age	16	3	1	2	4	3	2	31
	40.0%	14.3%	9.1%	13.3%	33.3%	42.9%	33.3%	27.7%
Abnormal serum	34	18	8	7	7	4	3	81
	85.0%	85.7%	72.7%	46.7%	58.3%	57.1%	50.0%	72.3%
Abnormal ultrasound	10	18	10	6	4	2	5	55
	25.0%	85.7%	90.9%	40.0%	33.3%	28.6%	83.3%	49.1%
High risk for thalassemia	3	2	1	2	1	–	1	10
	7.5%	9.5%	9.1%	13.3%	8.3%	0.0%	16.7%	8.9%
TORCH(+)	1	–	1	1	–	–	–	3
APH	1		1	1	–	–	–	3
Other indications	–	–	–	1	1	1	1	4

SCA: sex chromosomal aneuploid; APH: adverse pregnancy history

4. Discussion

Cordocentesis, chorionic villous sampling (CVS) and amniocentesis (AMC) of invasive procedures play crucial roles in detecting prenatal chromosome abnormalities. Different centers reported miscarriage rates from 0.2% to 3.6%[17, 18]. In the last decades, great efforts have been made to investigate new methods for noninvasive or rapid analysis of fetal chromosome abnormalities, such as noninvasive maternal serum prenatal genetic testing for fetal chromosome aneuploidies, fluorescence in situ hybridization (FISH), array comparative genomic hybridization (CGH) and multiplex quantitative fluorescent polymerase chain reaction (QF-PCR) analysis. Until now, these technologies have been wildly used in China. Cordocentesis is usually advised at 18–23 weeks[13]. and it can also be performed in the third trimester of pregnancy. For early diagnosis, if CVS or amniotic fluid culture is impossible, and cordocentesis would be a useful choice. Cordocentesis is also used to detect monogenic disease, hemoglobinopathies, immune deficiency syndromes and intrauterine infections. Cordocentesis is critically important for pregnant women of advanced maternal age in China, especially for those who could not receive the regular prenatal examinations in early pregnancy.

In the present study, with cordocentesis, 112 (4.8%) cases of fetal chromosome abnormalities were discovered, consistent with 1% to 5% reported by Driscoll *et al* [19], advanced age was the earliest indication for invasive procedures[20, 21], and frequencies of chromosome abnormalities were 1.4% in women aged 35–39 years and 3.5% in women aged 40 and above by another large study[22]. We have known that meiosis is very critical for advanced maternal age[23, 24]. Non-disjunction of chromosomes in maternal meiosis I is the origin of trisomy of the acrocentric chromosomes[25, 26]. while maternal meiosis II errors are the most common cause of 18-trisomy[27]. Extra chromosome 21 is the result of

non-disjunction during meiosis associated with advanced maternal age in either the egg or the sperm (standard trisomy 21) in approximate 95% of individuals[28, 29]. Here, we reported that cases of 21-trisomy, more than the cases of 18-trisomy and 13-trisomy, were discovered by advanced maternal age. This finding indicated that non-disjunction of metaphase chromosomes induced by advanced maternal age exerted a greater effect on chromosome 21 than chromosomes 13 and 18.

Our results showed that serum screening is superior to ultrasound screening in detecting 21-trisomy, however, ultrasound screening is superior to serum screening in detecting 13-trisomy. there is similar detection rate in 18-trisomy. 18-trisomy is associated with multiple severe structural abnormalities. ultrasound screening played a crucial rule in predicting chromosome abnormalities. Yang et al**[12]** and Ralston SJ[14] reported that 77%–100% trisomy 18 fetus and 20% DS fetus had major or structural anomalies that can be detected by ultrasound in second-trimester. All of the trisomy 18 fetus were discovered by abnormal single serum marker or multiple serum markers with low-risk compound. Therefore, abnormal single serum marker and multiple markers play important roles in predicting 21-trisomy and 18-trisomy. if an abnormal single serum marker or multiple markers were excluded, the positive screening rate of 21-trisomy and 18-trisomy were decreased, We also found that in the 21 cases of 18-trisomy fetus, 18 cases showed abnormal serum and ultrasound at the same time. Our results and reported studies both indicate that abnormal individual marker and multiple markers should be seriously regarded[29]. Any patient who had an abnormal serum marker, especially when combined with an abnormal ultrasound result, should be informed of fetal risk.

In California's triple-marker screening program, the detection rate for DS was 77.4%[30]. with a 5% to 9% false-positive rate. In the second trimester, the triple test for DS detection rate was 69% to 85%[31, 32]. In our study, the prenatal detection rate for DS was 85.0%, which is consistent with previous reports. More than 90% of chromosome disorders could be detected prenatally when ultrasound and biochemical markers were combined[33]. The detection rate for 21-trisomy reached 90% when maternal serum biochemistry test was used in conjunction with ultrasound scanning and maternal age[1]. In our study, 89.3% abnormal fetus were detected by using ultrasound and biochemical markers; 97.5% DS fetus were detected by combining maternal serum, ultrasound and maternal age. our screening rates were consistent with the reported ones.

Thalassemia has a high prevalence in south China; the risk of severe thalassemia was a main indication for cordocentesis[34]. In the present study, 17.5% patients (411/2342) showed a high risk for thalassemia. 40.1% (165/411) had abnormal serum screening, which indicated that thalassemia might influence the serum level of screening markers. Free β-hCG was significantly higher in women with fetal homozygous α-thalassemia-1 disease[35]. AFP and free β-hCG were significantly higher, whereas uE3 was lower in women with fetal Hb Bart's disease[36]. therefore, many pregnant women could not receive prenatal diagnosis during the early months of pregnancy,

In conclusion, when noninvasive prenatal examination is not used to be prenatal diagnosis, cordocentesis is a useful method for the detection of prenatal chromosome abnormalities. Cordocentesis is critically important for pregnant women with advanced maternal age, especially for those who could not receive regular prenatal examinations in China. In addition, cordocentesis rarely caused serious complications, such as placental abruption, and caused less bleeding, which had high accuracy and practicability in detecting chromosomal abnormalities in China.

Compared with sonographic markers, fetal structural abnormalities are more effective in predicting chromosomal abnormalities. In China, when serum screening is applied, a high-risk compound and an abnormal individual serum marker or multiple markers were of great value in predicting chromosomal disorders. Serum screening is an effective screening tool for detecting fetal 18-trisomy, 13-trisomy and 21-trisomy.

5. Conflict of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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References

[1] Liao C, Wei J, Li Q, Li L, Li J, Li D: Efficacy and safety of cordocentesis for prenatal diagnosis. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics 2006, 93(1):13-17.

[2] Tongsong T, Wanapirak C, Kunavikatikul C, Sirirchotiyakul S, Piyamongkol W, Chanprapaph P: Fetal loss rate associated with cordocentesis at midgestation. American journal of obstetrics and gynecology 2001, 184(4):719-723.

[3] Boupaijit K, Wanapirak C, Piyamongkol W, Sirichotiyakul S, Tongsong T: Effect of placenta penetration during cordocentesis at mid-pregnancy on fetal outcomes. Prenatal diagnosis 2012, 32(1):83-87.

[4] Harper J, Wells D, Simpson JL: Current controversies in prenatal diagnosis 4: preimplantation genetic screening should be routinely offered to all preimplantation genetic diagnosis cases. Prenatal diagnosis 2016, 36(1):25-28.

[5] Qi QW, Jiang YL, Zhou XY, Liu JT, Yin J, Bian XM: Genetic counseling, prenatal screening and diagnosis of Down syndrome in the second trimester in women of advanced maternal age: a prospective study. Chinese medical journal 2013, 126(11):2007-2010.

[6] Takyi A, Santolaya-Forgas J: Prenatal screening for chromosomal abnormalities in IVF patients that opted for preimplantation genetic screening/diagnosis (PGS/D): a need for revised algorithms in the era of personalized medicine. Journal of assisted reproduction and genetics 2017, 34(6):723-724.

[7] Hasegawa J: Ultrasound screening of umbilical cord abnormalities and delivery management. Placenta 2018, 62:66-78.

[8] Scott F, Coates A, McLennan A: Pregnancy outcome in the setting of extremely low first trimester PAPP-A levels. The Australian & New Zealand journal of obstetrics & gynaecology 2009, 49(3):258-262.

[9] Demirhan O, Pazarbasi A, Guzel AI, Tastemir D, Yilmaz B, Kasap M, Ozgunen FT, Evruke C, Demir C, Tunc E et al: The reliability of maternal serum triple test in prenatal diagnosis of fetal chromosomal abnormalities of pregnant Turkish women. Genetic testing and molecular biomarkers 2011, 15(10):701-707.

[10] Ferrier C, Dhombres F, Guilbaud L, Durand-Zaleski I, Jouannic JM: [Ultrasound screening for birth defects: A medico-economic review]. Gynecologie, obstetrique, fertilite & senologie 2017, 45(7-8):408-415.

[11] Tong H, Jin Y, Xu Y, Zou B, Ye H, Wu H, Kumar S, Pitman JL, Zhou G, Song Q: Prenatal diagnosis of trisomy 21, 18 and 13 by quantitative pyrosequencing of segmental duplications. Clinical genetics 2016, 90(5):451-455.

[12] Yang JH, Chung JH, Shin JS, Choi JS, Ryu HM, Kim MY: Prenatal diagnosis of trisomy 18: report of 30 cases. Prenatal diagnosis 2005, 25(2):119-122.

[13] Stembalska A, Slezak R, Pesz K, Gil J, Sasiadek M: Prenatal diagnosis--principles of diagnostic procedures and genetic counseling. Folia histochemica et cytobiologica 2007, 45 Suppl 1:S11-16.

[14] Ralston SJ, Craigo SD: Ultrasound-guided procedures for prenatal diagnosis and therapy. Obstetrics and gynecology clinics of North America 2004, 31(1):101-123.

[15] Estroff JA: Imaging clues in the prenatal diagnosis of syndromes and aneuploidy. Pediatric radiology 2012, 42 Suppl 1:S5-23.
[16] Filkins K, Koos BJ: Ultrasound and fetal diagnosis. Current opinion in obstetrics & gynecology 2005, 17(2):185-195.
[17] Alfirevic Z, Sundberg K, Brigham S: Amniocentesis and chorionic villus sampling for prenatal diagnosis. The Cochrane database of systematic reviews 2003(3):CD003252.
[18] Alfirevic Z, Navaratnam K, Mujezinovic F: Amniocentesis and chorionic villus sampling for prenatal diagnosis. The Cochrane database of systematic reviews 2017, 9:CD003252.
[19] Driscoll DA, Morgan MA, Schulkin J: Screening for Down syndrome: changing practice of obstetricians. American journal of obstetrics and gynecology 2009, 200(4):459 e451-459.
[20] Nicolaides KH: Screening for fetal aneuploidies at 11 to 13 weeks. Prenatal diagnosis 2011, 31(1):7-15.
[21] Zhang B, Lu BY, Yu B, Zheng FX, Zhou Q, Chen YP, Zhang XQ: Noninvasive prenatal screening for fetal common sex chromosome aneuploidies from maternal blood. The Journal of international medical research 2017, 45(2):621-630.
[22] Shimada S, Yamada H, Hoshi N, Kobashi G, Okuyama K, Hanatani K, Fujimoto S: Specific ultrasound findings associated with fetal chromosome abnormalities. Congenital anomalies 2009, 49(2):61-65.
[23] Allen EG, Freeman SB, Druschel C, Hobbs CA, O'Leary LA, Romitti PA, Royle MH, Torfs CP, Sherman SL: Maternal age and risk for trisomy 21 assessed by the origin of chromosome nondisjunction: a report from the Atlanta and National Down Syndrome Projects. Human genetics 2009, 125(1):41-52.
[24] Pradillo M, Santos JL: Genes involved in miRNA biogenesis affect meiosis and fertility. Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology 2018.
[25] Eggermann T, Nothen MM, Eiben B, Hofmann D, Hinkel K, Fimmers R, Schwanitz G: Trisomy of human chromosome 18: molecular studies on parental origin and cell stage of nondisjunction. Human genetics 1996, 97(2):218-223.
[26] Lin CY, Shukla A, Grady JP, Fink JL, Dray E, Duijf PHG: Translocation Breakpoints Preferentially Occur in Euchromatin and Acrocentric Chromosomes. Cancers 2018, 10(1).
[27] Ramesh KH, Verma RS: Parental origin of the extra chromosome 18 in Edwards syndrome. Annales de genetique 1996, 39(2):110-112.
[28] Morris JK, Alberman E, Mutton D, Jacobs P: Cytogenetic and epidemiological findings in Down syndrome: England and Wales 1989-2009. American journal of medical genetics Part A 2012, 158A(5):1151-1157.
[29] Mutton D, Alberman E, Hook EB: Cytogenetic and epidemiological findings in Down syndrome, England and Wales 1989 to 1993. National Down Syndrome Cytogenetic Register and the Association of Clinical Cytogeneticists. Journal of medical genetics 1996, 33(5):387-394.
[30] Kazerouni NN, Currier B, Lorey F, Roberson M: Triple-Marker Prenatal Screening Program for Chromosomal Defects. Obstetrics and gynecology 2009, 114(4):929.
[31] Lambert-Messerlian G, McClain M, Haddow JE, Palomaki GE, Canick JA, Cleary-Goldman J, Malone FD, Porter TF, Nyberg DA, Bernstein P et al: First- and second-trimester thyroid hormone reference data in pregnant women: a FaSTER (First- and Second-Trimester Evaluation of Risk for aneuploidy) Research Consortium study. American journal of obstetrics and gynecology 2008, 199(1):62 e61-66.
[32] Wald NJ, Rodeck C, Hackshaw AK, Rudnicka A: SURUSS in perspective. BJOG : an international journal of obstetrics and gynaecology 2004, 111(6):521-531.
[33] Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM: First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). Journal of medical screening 2003, 10(2):56-104.

[34] Liao C, Mo QH, Li J, Li LY, Huang YN, Hua L, Li QM, Zhang JZ, Feng Q, Zeng R et al: Carrier screening for alpha- and beta-thalassemia in pregnancy: the results of an 11-year prospective program in Guangzhou Maternal and Neonatal hospital. Prenatal diagnosis 2005, 25(2):163-171.

[35] Tongprasert F, Wanapirak C, Tongsong T: Maternal serum human chorionic gonadotropin and pregnancy-associated plasma protein-A in pregnancies with fetal homozygous alpha-thalassemia-1 disease. Prenatal diagnosis 2012, 32(7):700-702.

[36] Tongprasert F, Srisupundit K, Luewan S, Tongsong T: Second trimester maternal serum markers and a predictive model for predicting fetal hemoglobin Bart's disease. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet 2013, 26(2):146-149.