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Research Article

Instrument-Dependent or Instrument-Independent Indications and Prevalence of Chromosomal Abnormalities by Amniocentesis in China: An Analysis of 4146 Cases of Amniocentesis

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Abstract: Objective: There is a high incidence of birth defects in China, and prenatal diagnosis is an important method of intervention. This study aims to describe the clinical indications and cytogenetic results of amniocentesis cases in central China.

Methods: We retrospectively reviewed cases at the Maternal and Child Care Service Centre in Henan Province from January 2012 to December 2014. A total of 4497 at-risk mothers (risk factors: advanced maternal age, history of intrauterine fetal death or aborted fetuses, chromosomal abnormality in one of the parents, high-risk maternal serum screening results, and abnormal ultrasonographic findings in the first or second trimester) were recruited for amniocentesis (AS). The subjects included were between 11–14 and 18–22 weeks of gestation. All cases were divided into two groups based on instrument-independent or instrument-dependent indications.

Results: A total of 4146 cases were analyzed. Of these, chromosomal abnormalities were detected in 232 cases (5.6%), and autosomal aneuploidy, including trisomy 21 and trisomy 18, was found to be the most common (55.7%) chromosomal abnormality. The mean age of 29.94 years was not expected as all mothers older than 35 years old were routinely offered amniocentesis at the time of the study. Amniocentesis was carried out in 1711 cases because of instrument-independent indications, and 285 of these cases were diagnosed with chromosomal abnormality. In 2376 cases, amniocentesis was conducted because of instrument-dependent indications, and 176 of these were diagnosed with chromosomal abnormality. Thus, 5.6% of the cases were diagnosed with chromosomal abnormalities, and autosomal aneuploidy, including trisomy 21 and trisomy 18, were the most common chromosomal abnormalities detected in the present study

Conclusion: Our result indicated the significance of instrument-independent indications in the screening of chromosomal abnormalities, especially in developing areas. Birth defects may be reduced by paying more attention to the patients'history of medication.

Key words: Chromosome abnormality, Amniocentesis; Hint; High-risk pregnancy; Growth area

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0 Introduction

Amniocentesis is an invasive prenatal diagnostic test to detect chromosomal abnormalities in the fetus. Initially, the main purpose of prenatal diagnosis was to screen for Down's syndrome; however, due to the rapid development of serological screening and ultrasound examination and an increased proportion of patients with chromosomal abnormalities, a number of other chromosomal disorders can now be detected through amniocentesis[1]. Traditional noninvasive tests to screen for fetal aneuploidy are based on sonography and maternal biochemistry, and are associated with a detection rate of 50–95% with false positives encountered in 5% of all cases[2].

With the implementation of China's two-child birth policy, genetic counseling and prenatal care of pregnant women is likely to increase in the future. Therefore,

screening examinations such as amniocentesis will be of great importance in the second trimester. Furthermore, the cytogenetic detection of fetal chromosomal abnormalities using different clinical indicators is essential for prenatal genetic counseling.

Many tragedies can potentially be avoided if patients are given the opportunity to undergo amniocentesis. In recent years, the clinical application of prenatal diagnosis and selective abortion of fetuses with chromosome abnormalities have been increasing. Many literature reviews have shown the adverse effects associated with amniocentesis[3]. These adverse events include spontaneous abortion, infection, hemorrhage, fetal trauma, and Rh isoimmunization. Collectively, however, the incidence of these sequelae remains less than 1%[4-5]. Thus far, few Chinese studies have investigated the clinical and cytogenetic results of amniocentesis. Patients who are eligible to undergo this procedure have a basic ethical right to be properly educated about the risks and benefits of these tests[6]. The Royal College of Obstetricians and Gynaecologists (RCOG) has recommended that routine ultrasound exams should be performed at 18–20 weeks of gestation to facilitate the diagnosis of fetal abnormalities.

One of the main difficulties arising from routine anomaly scanning is the detection of minor sonographic abnormalities or "markers" such as choroid plexus cysts (CPCs), renal pelvic dilatation (RPD), echogenic bowel, mild cerebral ventriculomegaly, echogenic cardiac foci, and nuchal thickening[7]. The inability to adequately define risk means that health professionals cannot provide parents with adequate information upon which to base their decisions with regard to further management of the fetus. This can cause considerable anxiety[8], and likely result in greater costs, both economic and clinical. Therefore, there is an urgent need to assess the different clinical indications of chromosomal abnormalities obtained with and without instruments. Data should be collected prospectively from a large population with high-risk for aneuploidy with different risk factors to amniocentesis. However, the results of such a study could reduce the need for amniocentesis in the second trimester and reduce maternal anxiety in mothers with low-risk pregnancies. Counseling should be non-directive and include all relevant information[9]. Studies in the past have shown that adequate counseling before biochemical screening can help women decide whether to opt for the test, alleviate anxiety associated with a false positive result, and increase the appreciation of the possibility of a false negative result[10]. Prenatal cytogenetic detection of fetal chromosomal abnormalities is an effective method. Since the 1970s, amniocentesis has been a conventional means of prenatal testing in high-risk pregnancies with chromosomal abnormalities.

Currently, advanced maternal age (AMA), maternal serological screening (MSS), positive ultrasound screening in grade III, and different detection rates of chromosomal abnormalities are the main clinical indicators of amniocentesis in pregnant women. Ultrasound screening in the second trimester screening for fetal abnormalities provides reliable clues of underlying genetic disease. In recent years, use of ultrasound soft markers has effectively improved the detection rate of trisomy 21. Thus far, the distribution of chromosomal abnormalities and positive findings of ultrasound are unclear. Chromosomal abnormalities are the main cause of birth defects, and the rate of chromosomal abnormalities detected by positive ultrasound findings is 19.7% as an indicator before gestational age 22 weeks. Therefore, in this study, we retrospectively reviewed the clinical indications and cytogenetic results of amniocentesis cases in central China and investigated the diagnostic rates of different indicators used for prenatal screening.

1 Subjects and Methods

1.1 We conducted a retrospective study of 4146 cases of pregnant women who underwent amniocentesis for chromosome analysis at the Maternal and Child Care Service Centre in Henan Province between January 2012 and December 2014. Theprotocol was approved by the third affiliated hospitlal of Zhengzhou university medical ethics committee (NO 18) and informed consents were waived.

In this study, all the samples were derived from amniocentesis according to different clinical indicators, and genetic counseling was recommended in each case. It is worth mentioning that termination of the pregnancy was recommended for abnormal number of chromosomes. As polymorphisms result in structural abnormalities, doctors could give further advice based on the findings of the ultrasound screening, whether the polymorphism would result in an insignificant deformity or severely affect the fetus. Most of families decided not to terminate.

1.2 The position and orientation of the needle were determined by ultrasound guidance to avoid the placenta and umbilical cord, the location of the fetal limbs was determined, and 25 mL of amniotic fluid was collected under sterile conditions.

The obtained amniocytes were aseptically inoculated in GIBCO AmnioMAX-II and BIO-AMF-3, and incubated in a 5% CO^2 incubator at 37°C for 6–7 days. After culture for 1–2 days, colchicine was added to induce cell cycle arrest. G-banding chromosome preparation was used, and karyotype analysis was carried out using sample 2009 diagnostic criteria and divided into abnormal karyotypes, including autosomal aneuploidies, sex chromosome aneuploidy, balanced translocation, unbalanced translocation, polymorphism, and chimeras.

1.3 The following are clinical indicators for amniocentesis: (1) MSS, (2) AMA (≥ 35 years of age at the expected date of delivery), (3) adverse pregnancy history of aneuploidy, (4) chromosomal rearrangements in one of the parents, (5) abnormal ultrasound measurements detected before 22 weeks of gestation. The instrument-independent indications included (2), (3), and (4), and the instrument-dependent indications included (1) and (5).

1.4 The abnormal findings in prenatal ultrasound screening were as follows: 1) structural abnormalities according to "Obstetric Sonographer Qualification Exam of China Fetal Medicine Foundation," including the central nervous system, head, face, neck, heart, lung, urinary, reproductive, abdominal wall, abdominal organs, and limbs; 2) amniotic fluid volume; 3) ultrasound abnormal measurements , including cerebralventricle was 10mm or more, increased nuchal fold thickness was present if the thickness was 6mm or Mor e and the diagnosis of echogenic bowel required that this was of equal echogenicity to that of bone.The diagnosis of mild hydronephrosis was based on a minimum anteroposterior diameter of the renal pelvis,which varied between studies from 3mm to 4 or 5 mm.The definitions of short femur, short humerus and hypoplastic nasal bone were based on a cut-off of the respective bone length as a function of gestational age or biparietal diameter, and the cut-offs differed between studies[11].

2 Results

A total of 4497 amniotic fluid samples were obtained from January 2012 to December 2014 at our center, and 316 cases were excluded (Figure 1). The median age was 31 years, and the quartile ages were 27, 31, 37, and 47 years.

The clinical indicators are divided into five groups: AMA (26.2%, n=1087), maternal serum abnormalities (46.3%, n=1919), positive ultrasound findings (19.7%, n=813), adverse pregnancy history of aneuploidy (5.3%, n=220), and chromosomal rearrangements from one of the parents (0.6%, n=25) (Table 1).

A total of 232 patients were diagnosed with chromosomal abnormalities (5.6%), and autosomal aneuploidy, including trisomy 21 and trisomy 18, was the most commonly encountered chromosomal abnormality (55.7%). The distribution and detection rates of chromosomal abnormalities according to various clinical indicators for amniocentesis differed (Table 2). The most common chromosomal aberrations in familial propagation were balanced structural rearrangement (56%), and pregnancies continued. The pregnancies diagnosed with abnormal chromosome number (4%) or unbalanced structural rearrangement (28%) were terminated (Table 3). The most common aberrations detected in prenatal diagnosis were on chromosome 21 (42.71%), chromosome 18 (17.59%), chromosome 9 (8.04%) and sex chromosome (7.54%)(Table 4).

Table 1 Distribution of chromosomal abnormalities according to the clinical indications for amniocentesis

Indication for amniocentesis	Incidence (%)	Detection rate of abnormal karyotypes
Instrument-independent	1628/4146 (39.3%)	111/1628 (6.8%)
AMA and MSS negative	1188/4146 (28.7%)	74/1188 (6.2%)
AMA and MSS positive	265/4146 (6.4%)	22/265 (8.3%)
History of intrauterine fetal death or aborted fetuses	150/4146 (3.6%)	14/150 (9.3%)
Chromosomal abnormalities in one of the parents	25/4146 (0.6%)	1/25 (4.0%)
Instrument-dependent	2518/4146 (60.7%)	152/2518 (3.7%)
MSS positive and non-AMA	1788/4146 (43.1%)	50/1788 (2.8%)
Abnormal ultrasonographic findings in the first trimester	4/4146 (0.1%)	4/4 (100%)
Abnormal ultrasonographic findings in the second trimester	726/4146 (17.5%)	98/726 (13.4%)

AMA: Advanced maternal age; MSS: Maternal serum screening

Table 2 Chromosomal abnormalities from amniocentesis

Types	Cases	Frequency
Aneuploidy	140	60.3%
Autosomal aneuploidy	122	52.6%
Sex chromosome aneuploidy	18	7.8%
Structural abnormalities	41	17.7%
Balanced structural rearrangement	29	12.5%
Unbalanced structural rearrangement	1	0.4%
Chimera	11	4.7%
Polymorphism	42	18.1%
Others*	9	3.9%

*Including3 cases of ring chromosome,1 case of isochromosomes ,4 cases of multiple chromosome abnormalities and 1 case of rare chromosome abnormalities

Table 3 Ratios and outcomes of chromosomal aberrations in familial propagation

Types	cases	Ratio	Outcome
Abnormal chromosome number	1	4%	Termination
Structural abnormalities	21		
Balanced structural rearrangement	14	56%	Continuing
Unbalanced structural rearrangement	7	28%	Termination
Polymorphism	3	12%	Continuing
Total	25	100%	

Table 4 Chromosomal abnormalities detected in prenatal diagnosis

Chromosome	Cases	Ration
21#	85	36.6%
18#	35	15.1%
9#	16	6.9%
Sex chromosomes	48	20.7%
2#, 10#	5	2.2%
1, 11, 13, 14, 1522#	4	1.7%
7, 12#	3	1.3%
16#	2	0.9%
3, 4, 5, 6, 8, 17#	1	0.4%
19, 20#	0	0

3 Discussion

Most chromosomal abnormalities result in phenotypic abnormalities after birth, including congenital anomalies and mental abnormalities. As we all know, cytogenetic analysis of amniotic fluid is a reliable method for prenatal fetal testing. Here, 4497 cases of amniotic fluid cells were cultured and used for karyotype analysis in central China. The culture failure rate was 5.1%, which is slightly below the rate reported by Mademont-Soler et al.[12] In our study, the main reason for the clinical failure rate was the lack of patient information. Our data showed that the occurrence of chromosomal abnormalities was 5.6%, which is significantly higher than the results reported by Mademont-Soler et al., which studied a population of pregnant women in Spain. The reported differences in incidence between their study and ours may be because our study was based on different clinical indicators of high-risk pregnancy; furthermore, we classified polymorphisms as abnormal. In our study AMA accounted for 35% of cases of amniocentesis, which is significantly lower than the previously reported rate; this may be related to the prenatal screening system in central China.

Most research evidence suggests that different amniocentesis ratios and detection rates of chromosomal abnormalities can be obtained if different clinical indicators are used. Patients should be made aware of risks to make informed decisions; this will often alter their decisions[13]. Previously, AMA was the main clinical indicator of amniocentesis, because AMA is known to be associated with Down's syndrome. However, with the development of prenatal screening and ultrasound techniques, prenatal screening has gradually replaced AMA, and positive prenatal screening includes AMA among other relevant Down's syndrome indicators. Some studies using cytogenetic analysis of amniotic fluid have shown that the incidence of chromosomal abnormalities was 1–6.7%[14-15]. In our study, the incidence was found to be 5.6%, which is similar to the previously reported results.

The detection rates of chromosomal abnormalities using AMA, MSS, abnormal ultrasound findings, adverse pregnancy history of aneuploidy, and chromosomal rearrangement in one of the parents were 6.7, 10.4, 11.2, 8.6, and 16%, respectively. Clearly, chromosomal rearrangements in one of the parents had the highest detection rate, followed by abnormal ultrasound findings, and the detection rates with these indicators in our study were higher than those reported in Mademont-Soler et al.

Amniocentesis was the most accurate method to diagnose chromosomal abnormalities in utero. However, amniocentesis is associated with a risk of spontaneous abortion. This study indicated that fewer cases with instrument-independent indications underwent amniocentesis to rule out chromosomal abnormalities compared with cases with instrument-dependent indications. The instrument-independent indications screened more cases and had better accuracy to screen chromosomal abnormalities than instrument-dependent indications. Henan Province is a developing area. High-risk pregnancies undergo instrument-dependent examinations in rural areas. Therefore, the value of instrument-independent indications should be noticed in developing area.

In our study, the most common chromosomal abnormality was autosomal aneuploidy. Autosomal aneuploidy was the most commonly encountered chromosomal abnormality (52.6%). Trisomy 21 and trisomy 18 were the most common, followed by sex chromosome aneuploidy. In addition, the chromosomal abnormalities were most frequently detected in chromosome 21, 18, 9, and sex chromosomes in that order (Table 4). As detection of trisomy 21 is the main purpose of prenatal diagnosis, which can be diagnosed by ultrasound. In contrast, the detection rate of trisomy 13 has been reported to be less than 2.01%[16], which was detected in first trimester by ultrasound screening, pregnancies were terminated.

Our data revealed that the percentage of structural abnormalities was 17.7%, in which, balanced translocation was 0.7%, unbalanced translocation was 0.02%. This finding is similar to the study of Jacobs et al..[17]whose conclusions were similar.

In familial chromosomal abnormalities, 8 families decided to terminate due to positive ultrasound findings or abnormal number of chromosome. Among the other cases, those with structural abnormalities but with negative ultrasound findings opted to continue. Currently, genetic counseling is based on ultrasound screening for familial balanced translocation[18] and regular ultrasound screening and follow-up.

According to the abnormal ultrasound findings, the detection rate was 16.0%, which is less than that reported elsewhere. There were 4chromosomal abnormalities in 15 cases with nuchal thickening. It was 14% of chromosomal abnormalities Nicolaides et al.,[19] reported in birth defects. These disparities may be due to the different clinical indicators used and different ethnic backgrounds. It should be mentioned that only abnormal ultrasound findings were included in the prenatal test in their study (congenital malformations, intrauterine growth restriction, or both), whereas we included a broader spectrum of disease (congenital malformations, placental abnormalities, amniotic fluid volume abnormalities, and intrauterine growth restriction). On the other hand, we used soft markers for ultrasound screening in prenatal diagnosis, which were clinical indicators but not abnormalities

In developed countries, aneuploidy screening is adopted in the guidelines for prenatal diagnosis, followed by amniocentesis. Our data showed that the detection rate of amniocentesis was 6.7%, which was significantly lower than previously reported[20-22], because China is a developing country, and early fetal aneuploidy screening has not been fully promoted as a prenatal diagnostic tool. In our study, abnormal ultrasound findings and chromosomal rearrangements in one of the parents were associated with higher detection rates of chromosomal abnormalities, which is closely related to the rapid development of ultrasound technology in the past 10 years.

Our results demonstrated that ultrasound findings are a good indicator to detect fetal chromosomal abnormalities. Cytogenetic analysis of amniotic fluid is another reliable method of detecting fetal chromosomal abnormalities apart from Down's syndrome. As shown in Table 5, we found that ultrasound screening for fetal chromosomal abnormalities had a sensitivity and specificity of 41.7 and 81.5%, respectively. The kappa and concordance rates were 0.12 and 0.79, respectively; however, ultrasound could be used as diagnostic criteria for chromosomal abnormalities. The significant information yielded by ultrasound analysis can contributed to genetic counseling and help avoid unnecessary amniocentesis.

In our study, the detection rates of autosomal and sex chromosome aneuploidies were 55.7% and 8.2%, respectively, which is lower than that reported by Nishiyama et al.[23-24] Nowadays, chromosome breakpoints for accurate positioning by second-generation sequencing will contribute to detecting changes in genes and related sequences, and infer the genetic effects[25]. Nakata[26] assessed the tendency of genetic counseling for AMA from 2001 to 2007 (including MSS and invasive procedures), and found that effective prenatal screening and counseling decreased the number of invasive prenatal diagnostic

procedures. We studied the types and detection frequencies of chromosomal abnormalities in central China according to different clinical indicators and provided useful information for genetic counseling.

In a study in Japan, the birth rate of infants with trisomy 21 was found to have increased following the implementation of the policy againstlow fertility[27]. With the implementation of the two-child birth policy in China, it is likely that the numbers of AMA will increase rapidly, resulting in an increased need for genetic counseling.

This study has some limitations. First, it is a retrospective study, due to which data of outcomes of cases with fetal chromosomal abnormalities were unavailable. Second, there is no data of outcomes without any indications.

4 Conclusion

The incidence of chromosomal abnormalities in 4146 cases of amniocentesis was 5.6%. Amniocentesis was conducted in 1711 of the cases because of instrument-independent indications. Of these, 285 cases were diagnosed with chromosomal abnormality. Additionally, amniocentesis was conducted in 2376 cases because of instrument-independent indications; of these, 176 were diagnosed with chromosomal abnormality. The most common chromosomal abnormality was autosomal aneuploidy, including trisomy 21 and trisomy 18 (55.7%). Our result indicated the significant value of instrument-independent indications in screening of chromosomal abnormalities, especially in developing areas. Focusing on previous medication history may reduce birth defects.

References

[1] Shirley IM, Bottomly F, Robinson VP. Routine radiographer screening for fetal abnormalities by ultrasound in an unselected low risk population. *Br J Rad* 1992;65:564-9.

[2] Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* 2004; 191: 45–67.

[3] Robinson A, BowesW, Droegemueller W, et al: Intrauterine diagnosis: Potential complications. Am J Obstet Gynecol 116:937-941, 1973.

[4] Blajchman MA, Maudsley RF, Uchida I, et al: Diagnostic amniocentesis and fetal-maternal bleeding. Lancet 1:993-994, 1974.

[5] Wahlstrom J, Bartsch FK, Lundberg J. Prenatal chromosome determination: A study of 219 cases. Clin Genet 6:184-191, 1974.

[6] Lai FM, Ng CC, Yeo GS. Does maternal serum screening for Down syndrome induce anxiety in younger mothers? Singapore medical journal. 2004 Aug;45:375-378.

[7] Maclachlan N, Iskaros J, Chitty L. Ultrasound markers of fetal chromosomal abnormality: a survey of policies and practices in UK maternity ultrasound departments. Ultrasound in Obstetrics and Gynecology. 2000 May 1;15(5):387-390.

[8] Baillie C, Hewison J, Mason G. Soft-marker screening: Some women experience. Br J Obstet Gynaecol 1998; 105(Suppl.17):26.

[9] Elias S, Simpson JL. Genetic counselling. In: Simpson JL, Elias S, eds. Essentials of Prenatal Diagnosis. New York: Churchill-Livingstone, 1993; 3-13.

[10] Gekas J, Gondry J, Mazur S, Cesbron P, Thepot F. Informed consent to serum screening for Down syndrome; are women given adequate information? Prenat Diagn 1999;19:1-7.

[11] Agathokleous M, Chaveeva P, Poon LC, Kosinski P, Nicolaides KH. Meta-analysis of second-trimester markers for trisomy 21. Ultrasound in Obstetrics & Gynecology. 2013 Mar 1;41(3):247-261.

[12] Mademont-Soler I, Morales C, Clusellas N, Soler A, Sánchez A, Group of Cytogenetics from Hospital Clínic de Barcelona. Prenatal cytogenetic diagnosis in Spain: Analysis and evaluation of the results obtained from amniotic fluid samples during the last decade. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2011 Aug 31;157(2):156-160.

[13] Marteau TM, Cook R, Kidd J, Michie S, Johnston M, Slack J, et al. The psychological effects of false-positive results in prenatal sreening for fetal abnormality: A prospective study. Prenat Diagn 2000;20:705-708.

[14] Simpson NE, Dallaire L, Miller JR, Siminovich L, et al. (1976). Prenatal diagnosis of genetic disease in Canada: report of a collaborative study. Can. Med. Assoc. J. 23: 739-748.

[15] Tseng JJ, Chou MM, Lo FC, Lai HY, et al. (2006). Detection of chromosome aberrations in the second trimester using genetic amniocentesis: experience during 1995-2004. Taiwan J. Obstet. Gynecol. 45: 39-41.

[16] An N, Li LL, Wang RX, Li LL, Yue JM, Liu RZ. Clinical and cytogenetic results of a series of amniocentesis cases from Northeast China: a report of 2500 cases. Genetics and Molecular Research. 2015 Jan 1;14(4):15660-15667.

[17] Jacobs PA, Browne C, Gregson N, Joyce C, White H. Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding. Journal of medical genetics. 1992 Feb 1;29(2):103-108.

[18] Yakut S, Çetin Z, Şimşek M, Mendilcioğlu İİ, Toru HS, Berker Karaüzüm S, Lüleci G. Rare Structural Chromosomal Abnormalities in Prenatal Diagnosis; Clinical and Cytogenetic Findings on 10125 Prenatal Cases/Prenatal Tanlda Nadir Yaplsal Kromozom Anomaliler; 10125 Prenatal Olgunun Klinik ve Sitogenetik Bulgularl. Turkish Journal of Pathology. 2015 Jan 1;31(1):36-44.

[19] Nicolaides KH, Snijders RJ, Gosden CM, Berry C, Campbell S. Ultrasonographically detectable markers of fetal chromosomal abnormalities. Lancet 1992;340(8821):704–707.

[20] Lichtenbelt KD, Alizadeh BZ, Scheffer PG, Stoutenbeek

P, Schielen PC, Page-Christiaens LC, Schuring-Blom GH. Trends in the utilization of invasive prenatal diagnosis in The Netherlands during 2000–2009. Prenatal diagnosis. 2011 Aug 1;31(8):765-72.

[21] Nakata N, Wang Y, Bhatt S. Trends in prenatal screening and diagnostic testing among women referred for advanced maternal age. Prenatal diagnosis. 2010 Mar 1;30(3):198-206.

[22] Nishiyama M, Yan J, Yotsumoto J, Sawai H, Sekizawa A, Kamei Y, Sago H. Chromosome abnormalities diagnosed in utero: A Japanese study of 28983 amniotic fluid specimens collected before 22 weeks gestations. Journal of human genetics. 2015 Mar 1;60(3):133-137.

[23] Shaffer LG, Ledbetter DH, Lupski JR. Molecular eytogenetics of contiguous gene syndromes：Mechanisms and consequences of gene dosage imbalance. In: Scriver CR, Beaudet AL, Sly WS, et al Ed. Metabolic and molecular basis of inherited disease. New York: McGraw Hill. 2001. 1291-1324.

[24] Fantes JA, Boland E, Ramsay J, et al. FlSH mapping of de novo apparently balanced Chromosome rearrangements identifies characteristics associated with phenotypic abnormality. Am J Hum Genet, 2008, 82:916-926.

[25] Nakata N, Wang Y and Bhatt S (2010). Trends in prenatal screening and diagnostic testing among women referred for advanced maternal age. Prenat Diagn. 30: 198-206.

[26] Kajii T. Predicted prevalence of Down syndrome live births in Japan, 1970–2006.Am. J. Med. Genet. A 146A, 1387–1388 (2008).