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Cytogenetic Findings on 11,451 Cases of Amniocentesis in Hanoi, Vietnam

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Abstract

Since the 1960s, amniocentesis has become a routine procedure performed in prenatal diagnostic clinics. This study aims to depict the results of amniocentesis, the frequency of chromosomal abnormality and emphasize on amniocentesis indications. A retrospective study was conducted on 11,451 cases who were referred to the Prenatal Diagnosis Center, the National Hospital of Obstetrics and Gynecology, Hanoi, Vietnam from 2012 to 2016. The rate of chromosomal abnormality was 6.7%. The chromosomal aberration in the group with mother or father carrying balanced parental translocations accounted for the highest rate of 22.0%. Triploidy was 1.2%, autosomal chromosome aberration was 59.7%, sex chromosome was 8.3%, and structural rearrangements was 30.8%. Trisomy 21 was the most frequent disorder founded in abnormal ultrasound findings (47.4%), following by the advanced maternal age (44.1%). The large sample size of this study provided reliable evidence to support the development of prenatal counseling and pregnancy management programs.

Keywords: prenatal diagnosis, karyotype, amniocentesis indications

1. Introduction

Since the 1960s, amniocentesis (AS) has become a popular invasive test for those at high risks of genetic diseases (Jacobson & Barter, 1967; Wang & Cheng, 2009). Presently, amniocentesis is considered as a routine procedure at many prenatal diagnosis centers aiming to detect numeric and structural chromosomal disorders based on clinical findings and family history (Caron, Tihy, & Dallaire, 1999; Hook, Cross, & Schreinemachers, 1983). Pregnant women considered for AS are often of advanced age (over 34 years old) or under 34 in combination with positive maternal serum screening test at first or second trimester (Ferguson-Smith & Yates, 1984; Wang & Cheng, 2009). Many previously published studies have proposed other indications such as a study by Caron et al. on the Canadian population for 20 years looking at frequency of chromosomal aberations (Caron et al., 1999) and a study by Yi-Wen et al. in Taiwan for 30 years (Chang et al., 2012). The risk of chromosomal disorders was found to be higher in pregnant women with previous abnormal births, abnormal ultrasound findings, intra-uterine growth restriction (IUGR), or intra-uterine fetal death (IUFD). Tseng et al. demonstrated that the risk of chromosomal abnormalities was higher in the group with ultrasonographic malformations (Tseng et al., 2006). This study consists of 11,451 amniocentesis cases in the National Hospital of Obstetrics and Gynecology- the top-referral hospital in Hanoi, Vietnam from 2012 to 2016 in order to evaluate the various routine indications. The results of this study could hold valuable indications for genetic counseling.

2. Method

Clinical records were collected from amniocentesis database from the Center for prenatal diagnosis at the National Hospital of Obstetrics and Gynecology in Hanoi, Vietnam from 2012 to 2016. The indications of amniocentesis for cytogenetic analysis included: (1) previous abnormal chromosome birth, (2) previous congenital malformation births, (3) the history of miscarriage/stillbirth (more than 2 times), (4) parental carriers of chromosomal disorders, (5) positive maternal serum screening tests (cutoff with Down syndrome of 1/320), (6) advanced maternal age

(over 35 years old), and (7) abnormal ultrasound findings.

Two culture disks 4 ml Amniomax were prepared for each patient (Gibco, ThermoFisher Scientific). Then, the culture media was left in the disks for 10-13 days until the colonies were sufficient (about 30-40 colonies). Routine G-banding was performed for all specimens (Seabright, 1971).

Amniocytes were cultured in the genetic laboratory for about 2 weeks, then harvested for processing of cytogenetic results. Chromosomal analysis was performed in Carl-Zeiss automated microscopy system. All chromosomal disorders were classified into following categories: (1) autosomal chromosome aneuploidies, (2) sex chromosomal aneuploidies, (3) structural rearrangements (reciprocal translocation, Robertsonian translocation, deletion, inversion…). Frequencies of all observed disorders were calculated according to all indications.

2.2 Ethical Approval

This study was approved by the Ethical Committee of National Hospital of Obstetrics and Gynecology. Participants were informed of the study purpose, and were asked to give a written informed consent to confirm their participation. Participants could withdraw anytime and their information was kept confidential.

3. Results

Table 1. Case number and the rate of chromosomal disorders

Year	Number of cases	Abnormal number	Frequency of abnormality
2012	1447	87	6.0
2013	1830	91	5.0
2014	2701	151	5.6
2015	2558	209	8.2
2016	2915	232	8.0
Total	11451	770	6.7

The number of cases of amniocentesis indicated an increase from 1,447 cases in 2012 to 2,915 cases in 2016. In general, the number of chromosomal disorders was 770 cases (6.7%), ranging from 5.0% in 2013 to the peak of 8.2% in 2015 (Table 1).

Table 2. The prevalence of chromosomal aberrations by indication

Indications	N	%	N (Abnormalities)	% (Abnormalities)
Previous abnormal chromosome births	403	3.5	14	3.5
Previous congenital malformation birth	859	7.5	33	3.8
History of miscarriage/stillbirth	2392	20.9	171	7.1
Parental carriers of chromosomal disorders	123	1.1	27	22.0
Positive maternal serum screening tests	2354	20.6	107	4.5
Advanced maternal age	2419	21.1	68	2.8
Abnormal ultrasound findings	2901	25.3	350	12.1
Total	11451	100.0	770	6.7

Table 2 illustrates the prevalence of chromosomal aberrations of participants. The most common indication for AS was abnormal ultrasound findings (25.3%), followed by advanced maternal age (21.1%), history of miscarriage/stillbirth (20.9%), and positive maternal serum screening test (20.6%). Regarding chromosomal aberrations, the biggest proportion of chromosomal aberrations was found in parental carriers of chromosomal disorders (22.0%).

Table 3. Frequencies and types of chromosomal disorders by indications

	Previous abnormal chromosomes disorders N (%)	Previous congenital malformation birth N (%)	History of miscarriage/stillbirth N (%)	Parental carriers of chromosomal disorders N (%)	Positive maternal serum screening test N (%)	Advanced maternal age N (%)	Abnormal ultrasound findings N (%)	Total N (%)
Triploidy	0	0	2 (1,2)	0	2 (1,9)	0	5 (1,4)	9 (1,2)
Autosomal chromosomes disorders								**460 (59.7)**
Trisomy 21	5 (35.7)	13 (39.4)	66 (38.6)	3 (11.1)	30 (28.0)	30 (44.1)	166 (47.4)	313 (40.6)
Trisomy 18	0	2 (6.1)	27 (15.8)	0	3 (2.8)	1 (1.5)	73 (20.9)	106 (13.8)
Trisomy 13	0	0	4 (2.3)	0	1 (0.9)	0	11 (3.1)	16 (2.1)
Autosomal chromosome mosaicism	0	3 (9.1)	4 (2.3)	0	1 (0.9)	2 (2.9)	7 (2.0)	17 (2.2)
Other	0	1 (3.0)	4 (2.3)	0	0	0	3 (0.9)	8 (1.0)
Sex chromosomal disorders								**64 (8.3)**
45,X	0	1 (3.0)	2 (1.2)	0	2 (1.9)	1 (1.5)	9 (2.6)	15 (1.9)
47,XXY	0	0	3 (1.8)	1 (3.7)	3 (2.8)	4 (5.9)	8 (2.3)	19 (2.5)
47,XXX	0	0	2 (1.2)	0	0	1 (1.5)	4 (1.1)	7 (0.9)
47,XYY	0	1 (3.0)	4 (2.3)	0	2 (1.9)	0	4 (1.1)	11 (1.4)
Sex chromosome mosaicism	0	0	3 (1.8)	0	3 (2.8)	2 (2.9)	2 (0.6)	10 (1.3)
Other	0	0	1 (0.6)	0	0	0	1 (0.3)	2 (0.3)
Structural rearrangements								**237 (30.8)**
Reciprocal	2 (14.3)	4 (12.1)	6 (3.5)	9 (33.3)	12 (11.2)	1 (1.5)	7 (2.0)	41 (5.3)
Robertsonian	0	0	3 (1.8)	1 (3.7)	5 (4.7)	1 (1.5)	4 (1.1)	14 (1.8)
Inversion	0	4 (12.1)	12 (7.0)	4 (14.8)	14 (13.1)	7 (10.3)	15 (4.3)	56 (7.3)
Deletion	5 (35.7)	3 (9.1)	6 (3.5)	7 (25.9)	4 (3.7)	2 (2.9)	8 (2.3)	35 (4.5)
Duplication	2 (14.3)	1 (3.0)	7 (4.1)	2 (7.4)	4 (3.7)	4 (5.9)	4 (1.1)	24 (3.1)
Insertion	0	0	3 (1.8)	0	3 (2.8)	1 (1.5)	4 (1.1)	11 (1.4)
Marker	0	0	1 (0.6)	0	0	0	1 (0.3)	2 (0.3)
Polymorphism	0	0	11 (6.4)	0	18 (16,8)	11 (16.2)	14 (4.0)	54 (7.0)
Total	**14 (100)**	**33 (100)**	**171 (100)**	**27 (100)**	**107 (100)**	**68 (100)**	**350 (100)**	**770 (100)**

Of the 11,451 cases, 93.3% had normal karyotype (10,681 cases) and 6.7% had abnormalities (770 cases). In the group of chromosomal aberrations, 9 cases had triploidy (1.2%), 460 cases had autosomal chromosomes abnormality (59.7%), 64 cases had sex chromosomes aberrations (8.3%), and 237 cases had structural rearrangements (30.8%).

Triploidy (1.2%) was found more frequently in pregnant women with abnormal ultrasound findings (5 out of 9 cases), most common images found by ultrasound were enlarged, cystic placenta, and fetal structural anomalies (Table 3). The majority of numerical chromosome disorders was autosomal chromosomal trisomy (59.7%, 460/770). Trisomy 21 was the most common abnormality among all cases (40.6%), in which 47.4% was founded in abnormal ultrasound findings, and 44.1% was found in advanced maternal age. Trisomy 18 and 13 were found in 106 cases (13.8%) and 16 cases (2.1%), respectively. Additionally, 8 cases of rare aneuploidies were recorded in

this study: 1 case of trisomy 1, 2 cases of trisomy 22, 1 case of trisomy 8, 1 case of trisomy 9, 1 case of trisomy 12, 1 case of trisomy 9, and 1 case of monosomy 10. In cases of sex chromosome aneuploidies, monosomy X found in 15 cases (1.8%), 19 cases were 47,XXY (2.5%), 11 cases were 47,XYY (1.4%), 10 cases were mosaicism (1.3%), 7 cases were 47,XXX (0.9%). Total structural rearrangements were 237 cases (30.8%). Inversion was the most frequent aberration detected with chromosome structure (7.3%), followed by polymorphism (7.0%) and reciprocal translocation (5.3%). Most of the structural rearrangement cases found in abnormal ultrasound findings and history of miscarriage/stillbirth.

4. Discussion

The number of amniocentesis cases and chromosomal disorders were gradually increased each year, ranging from 5.0% in 2013 to the peak of 8.2% in 2015. The overall rate of chromosomal disorders was 6.7%. While this result was lower than the result from a study in Turkey conducted on 13,466 amniocentesis cases (Özer, Ünsal, Ayvaz, Şen, & Baltacı, 2016), it was higher than those of other studies which ranged from 2.7% to 3.6% (Chang et al., 2012; Han et al., 2008). Presumably, during the Vietnam War, prior generations were exposed to the large amount of toxic chemicals (Field & Kerr, 1988), this may lead to the likelihood of chromosomal defects in the offspring. Additionally, as the Prenatal Diagnosis Center at the National Hospital of Obstetrics and Gynecology in Vietnam is the top-referral for prenatal diagnosis, this may explain the high number of high-risk cases in comparison with other facilities.

AS indications based on abnormal findings during prenatal examinations included: (1) previous abnormal chromosome disorders, (2) previous congenital malformation birth, (3) history of miscarriage/stillbirth, (4) parental carriers of chromosomal disorders, (5) positive maternal serum screening tests, (6) advanced maternal age, (7) abnormal ultrasound findings. Amniocentesis involves certain risks for pregnant women such amniorrhea (the absence of menstrual periods), miscarriage, fetal death. Thus, indications for amniocentesis must be under consideration of both risks and benefits.

Abnormal ultrasound finding was the most common amniocentesis indication which accounted for 25.3% and the rate of chromosomal abnormalities in this group was 12.1%. Previous studies revealed the various rates of chromosomal abnormalities ranging from 4% to 15.5% (Chang et al., 2012; Ocak, Ozlu, Yazicioglu, Ozyurt, & Aygun, 2014; Özer et al., 2016). Although 21.1% participants were in advanced maternal age, only 2.8% found with chromosomal disorders. It is suggested that the risk of chromosomal nondisjunction is correlated with maternal age (Hassold & Hunt, 2009). This was consistent with a large report in Europe with 52,965 pregnant women (Ferguson-Smith & Yates, 1984). In this study, 123 couples (1.1%) were carriers for chromosomal disorders, mostly reciprocal translocation and the rate of abnormal offspring this pregnancy peaked at 22.0%. A previously published study found 1.5% couples with chromosomal aberrations and 11.51% with chromosomal disorders (Chang et al., 2012). Furthermore, Zepney Ocak et al. (2014) found that 100% offspring had chromosomal rearrangement among parental translocation carriers (Ocak et al., 2014).

In our present database, couples with previous miscarriage and/or stillbirth were accounted for 20.9% and the rate of chromosome abnormalities in this group was 7.1%. This figure was even higher than couples with the history of abnormal chromosome disorders and congenital malformation (3.5% and 3.8%, respectively). In developing countries, miscarriage and/or stillbirth are highly prevalent due to the poverty and the lack of education (Aminu et al., 2014). In addition, in Vietnamese culture, sexual health topics are often avoided and not up for discussion (Pham et al., 2012). As a result, there is a potential gap in sexual and reproductive health knowledge among Vietnamese women. Thus, it is important to raise the knowledge and awareness regarding sexual and reproductive health among Vietnamese women. We recommend that couples with recurrent miscarriage and/or stillbirth should consider screening for indicated karyotype carriers.

Types of chromosomal abnormalities included triploidy (1.2%, 9/770), autosomal chromosome disorders (59.7%, 460/770), sex chromosome disorders (8.3%, 64/770), structural rearrangements (30.8%, 237/770) which concurred with previous studies (Chang et al., 2012; Han et al., 2008; Ocak et al., 2014). Trisomy 21 was most frequent, found in 40.6% of the total abnormal cytogenetic findings; and among those, 47.4% was with morphological malformations found by ultrasound. The frequency of trisomy 21 in the second trimester was higher because another small proportion of trisomy 21 had been lost spontaneously *in utero* before delivery (Hook, 1983). Zhang et al recorded that the frequency of trisomy 21 was about 35.6% of all chromosomal detected by karyotyping (Zhang et al., 2011). Similarly, the study by Han et al, which examined 31,615 high-risk pregnancies, reported 36.9% was trisomy 21 (Han et al., 2008). Our study results confirmed that trisomy 21 was the most frequent chromosomal aberration in pregnancy.

The most common structural aberration was inversion (7.3%), followed by reciprocal (5.3%), and deletion (4.5%).

The majority of those disorders found with the history of miscarriage and/or abortion and abnormal ultrasound findings. This pattern was consistent with a study by Ocak et al in 2013 (Ocak et al., 2014). Reciprocal translocations from parental carriers increase the risk of miscarriage and implantation failure. Genetic material unbalance causes congenital malformations, intra-uterine growth restriction which can be detected via ultrasound. In such condition, preimplantation genetic diagnosis (PGD) was appropriate. Studies have shown that clinical pregnancy rate per oocyte increased from 29.0% to 38.0% and the rate of miscarriage decreased from 92.0% to 12.5% (Dietterich, Check, Choe, Nazari, & Lurie, 2002). Moreover, PGD could be indicated for couples suspected with genetic carriers aiming at enhanced genetic quality and avoiding spreading mutations in the population.

In this study, 54 cases of polymorphism were detected (7.0%). Among those, 8 cases with increased nuchal translucency and 8 cases of abnormal ultrasound, other cases were positive with maternal serum screening test. Polymorphisms were possibly STR, SNP or repeat of the satellite. However, there was no reason for inherited conditions. A recent study raised the hypothesis which chromosomal polymorphism at heterochromatin was responsible for decreasing reproductive function and recurrent miscarriage among infertile couples (Madon, Athalye, & Parikh, 2005).

5. Conclusion

Our study indicated that the overall rate of chromosomal disorders, in which autosomal chromosome disorders and structural rearrangements were the most frequently found among chromosomes abnormal cases. Regarding amniocentesis indication, abnormal ultrasound finding was the most common reason for amniocentesis test. This study provides reliable evidence to develop prenatal counseling and pregnancy management.

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Data Availability

The data could be obtained by contacting corresponding author.

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Competing Interests Statement

No competing interests were disclosed.

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