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Effect of First-trimester Ultrasound Examination for Chromosomal Aberrations in Women Undergoing Amniocentesis

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Abstract

Objective: The purpose of this study was to assess the effect of first-trimester ultrasound examination for chromosomal aberrations in women who underwent amniocentesis.

Methods: To evaluate trends in the indications of amniocentesis and the number of chromosomal aberrations, we reviewed all amniotic fluid samples from genetic amniocentesis processed by the Tama-Nagayama Hospital of Nippon Medical School from 1991 through 2005. The referral indications included first-trimester abnormal ultrasound finding.

Results: A total of 1,054 women underwent genetic aminiocentesis in the first- to early second-trimester, and 1,063 amniotic samples were processed. The overall rate of chromosomal aberrations was 3.3% (35 of 1,063 samples), and the rate of aberrations remained unchanged during the study period. The number of cases with abnormal ultrasound finding increased from 5 (1.1%) in the first 5-year period to 46 (19.4%) in the last 5-year period ($p<0.01$). In contrast, the number of amniotic fluid samples per year tended to decline during the study period.

Conclusion: First-trimester ultrasound examination had a significant effect on our amniocentesis cases. The application of first-trimester ultrasound examination may be associated with a lower rate of invasive genetic testing.

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Key words: prenatal diagnosis, amniocentesis, chromosome aberrations, ultrasonography

Introduction

The possibility of chromosomal aberration is present in every pregnancy. Prenatal diagnosis of chromosomal aberration necessitates invasive testing, by amniocentesis or chorionic villous sampling, which is associated with an approximately 1% risk of miscarriage[1]. Consequently, invasive testing in general is offered only to women considered to be at increased risk of carrying a fetus with Down syndrome or other significant chromosomal aberrations[2]. Most of the women who are offered invasive testing, however, are concerned about its potential risks: the risk of procedure-related miscarriage, the risk of anxiety associated with testing, and the risk of the financial costs of the procedure[3].

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Recently, most pregnant women undergo prenatal ultrasound examinations and measurement of maternal serum markers as a routine part of antenatal care. In particular, nuchal translucency (NT), the sonographic measurement of the posterior nuchal skin, is widely used as a screening tool for fetal aneuploidy[1,4-6]. The traditional indicator of genetic risk is maternal age, with which invasive testing in 5% of the population identifies approximately 30% of fetuses with trisomy 21[7]. There is now extensive evidence that ultrasound examination, combined with maternal serum biochemical testing at 11 to 13 weeks of gestation, can identify more than 95% of fetuses with major chromosomal abnormalities[7]. Although ultrasound has the potential to improve the performance of Down syndrome screening programs, it can also cause harm by prompting unnecessary medical intervention, anxiety related to false-positive findings, and false reassurance to women with affected pregnancies who may be dissuaded from undergoing a diagnostic test because of a normal ultrasound result[8].

In this study, we investigated our cases of genetic amniocentesis for 15-years and assessed the effect of first-trimester ultrasound examination for chromosomal aberrations in women who underwent amniocentesis.

Materials and Methods

From 1991 through 2005, 1,054 women, including 1,045 with singleton pregnancies and 9 with twin pregnancies, underwent genetic aminiocentesis in the first to early second trimester (12 to 19 week of gestation), and 1,063 amniotic samples were processed in our hospital. For the sake of convenience, we divided the 15-year study period into three 5-year intervals: period A from 1991 through 1995, period B from 1996 through 2000, and period C from 2001 through 2005. We reviewed cases from medical records and examined the period of testing, indications for amniocentesis, maternal age, gestational age at amniocentesis, and fetal karyotype. This study was approved by the institutional review board.

Amniocentesis was performed with written informed consent after genetic counseling, and fetal karyotype was analyzed by G-banding. In cases of mosaicism with normal and abnormal cells, the type of abnormal cell is stated. We regarded [46,inv (9) (p11q13)] as a normal variant.

In many cases, more than one indication for amniocentesis was present. For simplicity, we reduced multiple referral indications to a single indication using the following priority order: 1) abnormal ultrasound findings, 2) positive screening for maternal serum marker, 3) chromosomal translocation carrier, 4) pervious birth with chromosomal aberration, 5) advanced maternal age of 35 years or older, 6) other heredity disease (s) (e.g., X-linked disease and testicular feminization), and 7) "other" cases including client's request on the basis of anxiety, history of habitual abortion, and relatives with Down syndrome.

In our hospital, all pregnant women underwent ultrasound examination as part of routine obstetric care. However, we did not perform advanced first-trimester genetic ultrasound screening routinely. If the findings of routine ultrasound examinations were abnormal, trained obstetricians performed a more detailed ultrasound examination to confirm the abnormal findings. To detect fetal NT, the thickness of the subcutaneous fluid-filled space between the fetal skin and the soft tissue overlying the cervical spine was measured on a sagittal section with abdominal or transvaginal ultrasound examination at 10 to 14 weeks' gestation.

We did not actively offer maternal serum marker testing for pregnant women. Maternal serum marker testing was performed only for women who requested the testing and consented after genetic counseling.

We analyzed continuous data with the Kruskal-Wallis test, and group data were analyzed with the likelihood ratio method. For tests of significance, a P value of less than 0.05 was considered to indicate significance.

Results

The characteristics of subjects are shown at

Table 1 Characteristics

Indication [a]	n	Maternal age (year) [b] [c]	Gestational age (day) [b] [c]	Chromosomal aberrations [d]
maternal age	794	37.9 ± 2.3	106.8 ± 7.0	19/798 (2.4%)
abnormal ultrasound	70	30.6 ± 4.4	109.3 ± 6.6	11/70 (15.7%)
maternal serum marker	11	36.0 ± 3.4	122.5 ± 3.6	1/11 (9.1%)
translocation carrier	6	31.3 ± 4.0	107.0 ± 5.8	3/6 (50.0%)
heredity disease	8	31.8 ± 4.5	110.6 ± 8.6	0/8 (0.0%)
past history	46	32.3 ± 4.8	106.0 ± 5.0	0/46 (0.0%)
other	119	31.2 ± 2.9	107.3 ± 7.6	1/124 (0.8%)
total	1,054	36.3 ± 3.9	107.1 ± 7.2	35/1,063 (3.3%)

a) Maternal age means advanced maternal age.
Abnormal ultrasound means abnormal ultrasound findings.
Translocation carrier means carrier of chromosomal translocation.
Past history means subject's past history of giving birth with chromosomal aberration.
b) Value are given as mean ± SD
c) $p<0.01$ analysed by Kruskal-Wallis test
d) $p<0.01$ analysed by likelihood ratio method

Table 2 Changes in the Indication of amniocentesis in 15 years

Indication [a]	Period A	Period B	Period C	All periods
	1991 ～ 1995 n=463	1996 ～ 2000 n=354	2001 ～ 2005 n=237	1991 ～ 2005 n=1,054
maternal age	367 (79.3%)	267 (75.4%)	160 (67.5%)	794 (75.3%)
abnormal ultrasound	5 (1.1%)	19 (5.4%)	46 (19.4%)	70 (6.6%)
maternal serum marker	0 (0%)	6 (1.7%)	5 (2.1%)	11 (1.0%)
translocation carrier	1 (0.2%)	4 (1.1%)	1 (0.4%)	6 (0.6%)
heredity disease	4 (0.9%)	3 (0.8%)	1 (0.4%)	8 (0.8%)
past history	16 (3.5%)	18 (5.1%)	12 (5.1%)	46 (4.4%)
others	70 (15.1%)	37 (10.5%)	12 (5.1%)	119 (11.3%)

$p<0.01$: analysed by likelihood ratio method
a) Maternal age means advanced maternal age,
abnormal ultrasound means abnormal ultrasound findings,
translocation carrier means carrier of chromosomal translocation,
past history means past history of giving birth with chromosomal aberration.

Table 1. In the 1,054 cases, the mean maternal age was 36.3 ± 3.9 years and the gestational age at amniocentesis was 107.1 ± 7.2 days. Twin pregnancy was observed in nine cases. Finally, 1,063 amniotic fluid samples were analyzed. The number of amniocentesis samples decreased from 468 in period A to 239 in period C.

The indications for amniocentesis included "advanced maternal age" in 794 cases, "abnormal ultrasound findings" in 70 cases, "positive maternal serum markers" in 11 cases, "translocation carrier" in 6 cases, "past history" in 46 cases, "heredity disease" in 8 cases, and "other" in 119 cases.

The detection rate of chromosomal aberrations was 3.3% (35 of 1,063 samples). Higher rates of chromosomal abnormality were found in cases with translocation carriers (3 of 6 cases) and abnormal ultrasound findings (11 of 70 cases). All abnormal karyotypes obtained from translocation carriers were balanced translocatons that were identical to those of each parent. An abnormal result from cases positive for "maternal serum markers" was obtained in only 1 case (detection rate. 9.1%) with 46, XYY.

Table 2 shows the changes in the indications for amniocentesis during the study period. Annual changes in the indications were characterized by cases of advanced maternal age and of abnormal ultrasound findings. During the study period, the

Table 3 Changes in the result of amniocentesis in 15 years

Result	Period A	Period B	Period C	All periods
	1991 ～ 1995 n=468	1996 ～ 2000 n=356	2001 ～ 2005 n=239	1991 ～ 2005 n=1,063
Normal	452 (96.6%)	346 (97.2%)	230 (96.2%)	1,028 (96.7%)
Abnormal	16 (3.4%)	10 (2.8%)	9 (3.8%)	35 (3.3%)
Balanced Translocation	5 (1.1%)	2 (0.6%)	2 (0.8%)	9 (0.8%)
Inversion	1 (0.2%)	0 (0.0%)	1 (0.4%)	2 (0.2%)
Trisomy13	1 (0.2%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
Trisomy18	2 (0.4%)	2 (0.6%)	2 (0.8%)	6 (0.6%)
Trisomy21	4 (0.9%)	4 (1.1%)	3 (1.3%)	11 (1.0%)
46, XX/46, XY	1 (0.2%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
Turner Syndrome	1 (0.2%)	1 (0.3%)	1 (0.4%)	3 (0.3%)
47, XXY	1 (0.2%)	0 (0.0%)	0 (0.0%)	1 (0.1%)
47, XYY	0 (0.0%)	1 (0.3%)	0 (0.0%)	1 (0.1%)

N.S.: analysed by likelihood ratio method

Table 4 The relation between karyotype and abnormal ultrasound findings in 15 years

US [a] findings	Period A		Period B		Period C		All periods	
	1991 ～ 1995 n=5		1996 ～ 2000 n=19		2001 ～ 2005 n=46		1991 ～ 2005 n=70	
	karyotype		karyotype		karyotype		karyotype	
	abnormal	normal	abnormal	normal	abnormal	normal	abnormal	normal
NT [b]	1	0	3	12	5	35	9	47
Hydrops Fetalis	2	0	0	0	0	2	2	2
other findings [c]	0	2	0	4	0	4	0	10

N.S.: analysed by likelihood ratio method
a) US means ultrasound
b) NT means nuchal translucency.
c) Other findings include fetal growth restriction, limb anomaly, abdominal anomaly, hydrocephalus and placental abnormality.

percentage of cases with abnormal ultrasound finding increased from 1.1% (5 of 437 cases) in period A to 19.4% (46 of 237 cases) in period C. In contrast, the percentage of cases with advanced maternal age declined from 79.3% (367 of 463 cases) in period A to 67.5% (160 of 237 cases) in period C. The number of cases with positive maternal serum markers was 0 in period A, 6 in period B, and 5 in period C.

Changes in the results of karyotype testing are shown at **Table 3**. Of 1,063 samples collected over 15 years, 1,028 showed a normal karyotype, and 35 showed an abnormal karyotype (3.3%). The total detection rate of abnormal karyotypes did not differ significantly (3% to 4%) throughout the study period.

Table 4 shows the relationship between ultrasound findings and karyotype abnormalities. Abnormal ultrasound findings were classified into three categories: hydrops fetalis, NT, and other findings (fetal growth restriction, limb anomaly, abdominal anomaly, hydrocephalus, placental abnormality). The number of cases of hydrops fetalis or of other findings showed no changes through the 3 survey periods. On the other hand, the number of NT cases increased from 1 in period A to 40 in period C. Chromosomal aberrations were found only in cases of NT and of hydrops fetalis. In particular, chromosomal aberrations were detected in 16.1% (9 of 56) of NT cases. The type of abnormal karyotype in NT varied: there were 2 cases of trisomy 18, 3 cases of trisomy 21, 3 cases of Turner syndrome, and 1 case of mosaicism with a normal karyotype and *de novo* balanced translocaton [46/46,t (4;18) (q31.1;q22)].

Discussion

Ultrasound examination had a significant effect on our amniocentesis cases. During period A (1991 through 1995), the percentage of cases with abnormal ultrasound findings was only 1.1%. The percentage of cases with abnormal ultrasound findings increased to 19.4% in period C (2001 through 2005). NT is most responsible for the increase in the number of amniocentesis cases indicated by abnormal ultrasound findings. At present, NT measurement has become the most common method for fetal chromosomal screening because of its high detection rate[9–12]. However, the overall sensitivity of NT finding is too low for it to be a practical screening test for trisomy 21[8]. Wapner et al[13] have shown that first trimester screening for trisomy 21 and trisomy 18 that combined NT thickness with maternal age, levels of pregnancy-associated plasma protein A and free beta human chorionic gonadotropin is accurate and efficient in clinical practice. This screening approach could identify 85.2% of 61 cases of Down syndrome with a false-positive rate of 9.4% and could identify 90.9% of 11 cases of trisomy 18 with a false positive rate of 2%. Indeed, most pregnant women undergo NT measurement as a routine part of antenatal care in the United States[1].

In addition to NT, there are other ultrasound findings associated with fetal chromosomal aberrations, such as nasal bone defects[14], and Doppler study of ductus venous[15]. However, these assessments are not commonly used for first-trimester screening, because they are time-consuming and require skilled operators[7]. For this reason, we did not screen for nasal bone defects or ductus venous in our study. Consequently, the only ultrasound findings related with chromosomal aberrations were NT and hydrops fetalis.

This study included 11 cases of amniocentesis with positive maternal serum markers during the past 15 years. In North America and the United Kingdom, the American College of Obstetricians and Gynecologists[4], the Canadian Task Force on the Periodic Health Examination[5], and the National Collaborating Centre for Women's and Children's Health[6] offered serum marker screening to all pregnant women for neural tube defects and trisomies 21 and 18. In contrast, the Japanese Ministry of Health, Labour and Welfare[16] issued its deliberate opinion for maternal serum screening in 1999 and stated that a physician is not required to actively give pregnant women information about maternal serum screening and should not recommend this examination. In fact, Matsuda and Suzumori[17] have reported that few pregnant women are being offered maternal serum marker screening in Japan.

In our study, the total number of amniocentesis cases tended to decline over 15 years. This trend was affected by the decrease in cases of advanced maternal age. Some reports have found that the use of these genetic markers reduces the frequency of chorionic villous sampling and of amniocentesis for normal pregnancies and prevents potential procedural fetal losses during normal pregnancy[3,11,18,19]. Chasen et al have reported that the use of NT is not associated with an increase in invasive testing in women older than 35 years[11]. In contrast, Cheffins et al[20] have reported that maternal serum marker testing increases the frequency of invasive prenatal testing. We perform ultrasound examination as a routine part of obstetric care for all pregnant women. However, we perform serum marker testing only if the women has requested it. Under these circumstances, women older than 35 years who have normal ultrasound results and a low risk for chromosomal aberrations may avoid amniocentesis. For these reasons, we speculate that the active application of ultrasound examination and the passive use of maternal serum marker testing have reduced the number of our amniocentesis cases.

In conclusion, first-trimester ultrasound examination has a significant effect on our amniocentesis cases. Although further examination will be needed, general application of first-trimester ultrasound examination as observed in our study may be associated with a lower rate of invasive genetic testing.

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