**ORIGINAL ARTICLE**

# A four - year retrospective study of amniocentesis: one centre experience

Daniilidis A, Karydas H, Zournatzi V, Tantanasis T, Giannoulis C, Tzafettas J

2nd Department of Obstetrics and Gynecology, Hippokratio University Hospital, Thessaloniki, Greece

**Abstract**

**Aim**: Monitor the performance of the amniocentesis procedure for prenatal diagnosis and particularly the acquisition of results (time to get, success in getting them).

**Materials and Methods:** This is a retrospective review of case notes of all pregnant women undergone amniocentesis in our department during the period 2002-2005. Two main operators performed the procedure, using 22 gauze needle usually and 20 gauze should longer needle was needed. Sevendy three patients undergone amniocentesis. The reasons for having this procedure were: increased risk for Down syndrome in 68% (50/73), maternal request in 24% (18/73), suspicious ultrasound findings in 4% (3/73) and family history in 3% (2/73). Maternal age ranged from 20 to 45 years and the gestation time that amniocentesis was performed was 15 to 23 weeks. Fluorescence in situ hybridization (FISH) and culture were used in order to obtain karyotype results.

**Results:** In 92% (67/73) of cases one needle pass was needed. FISH and culture were performed in 96% (70/73) of cases, FISH only in 3% (2/73) and culture only in 1% (1/73). The chromosome results were normal in 93% (68/73) of cases, Down's syndrome in 4% (3/73) and Edwards syndrome in 3% (2/73). The outcome of pregnancies was: live births in 89% (65/73), stillbirths at 32 weeks and 35 weeks in 3% (2/73), miscarriages in 1% (1/73 at 19 weeks, 3 weeks after the amniocentesis), terminations in 7% (5/73, due to chromosomal abnormalities). Sixty one women delivered at term (84%) and 6 women (8%) delivered preterm.

**Conclusions:** The post amniocentesis rate of miscarriage is calculated at 1% in our centre. FISH analysis can relieve stress of couples by reducing the waiting time for results. Hippokratia 2008; 12 (2): 113-115

**Key words:** amniocentesis, chromosomal abnormalities, pregnancy

**Corresponding author:** Daniilidis A, 9 Smirnis, 56224, Thessaloniki, tel: 00306932211395 Fax: 00302310559711, e-mail ange1972@otenet.gr

Amniocentesis was first introduced into obstetric practice as a mean of detecting the severity of rhesus (Rh) isoimmunisation about 50 years ago[1]. Invasive prenatal diagnosis and particularly amniocentesis was introduced into clinical practice in the 1970s, as the mid-trimester diagnostic investigation of choice. Primarily it is being carried out in order to detect Down syndrome, by obtaining fetal cells for cytogenetic analysis. Due to the fact that any invasive procedure is associated with a risk of miscarriage[2] it is currently applied only to a small group of women which are in a higher risk of having an offspring with a chromosomal defect in comparison to the general population. In order to determine this "high risk" group some screening approaches have been developed during the years. Maternal age, with a cut-off of 35 years was the most common indication in the past. Also the history of chromosomal abnormality was also considered to be a valid indication at that time. Preliminary screening tests based on maternal age, maternal serum biochemical parameters, and ultrasound measurements of the fetus, have been widely adopted in order to select the high risk group which in fact has an indication for amniocentesis nowadays[3,4].

Amniocentesis remains the most common invasive prenatal diagnostic procedure today[3]. However, specific standards should be followed in order to ensure that damage to the pregnancy is limited[5].The aim of this retrospective study is to monitor the performance of the amniocentesis service for prenatal diagnosis in our Department and particularly the acquisition of results (time to get, success in getting them).

## Materials and Methods

This is a retrospective study, for a four years period (2002-2005), of case notes of all pregnant women who attended our department, in order to have amniocentesis. During this period, two main operators performed the procedures, using 22 gauze needles usually, but sometimes using 20 gauze should longer needle was needed. Seventy three patients undergone amniocentesis (Figure 1). The reasons for having this procedure were:

1. Increased risk for Down's syndrome in 68% (50/73) according to first trimester screening test (maternal age + nuchal translucency + β-hCG + PAPP-A [pregnancy associated plasma protein A]).
2. Maternal request in 24% (18/73).
3. Suspicious ultrasound findings in 4% (3/73).
4. Family history in 3% (2/73).

We used fluorescence in situ hybridization and culture in order to obtain results from all specimens. FISH and culture were performed in 96% (70/73) of cases, FISH only in 3% (2/73) and culture only in 1% (1/73).

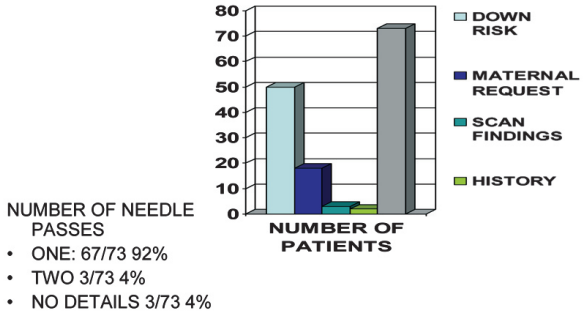
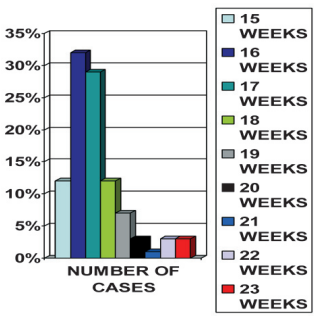



**Figure 1.** Number of patients having amniocentesis and number of needle passes.

## Results

Maternal age ranged from 20 to 25 years in 4% (3/73), 26 to 30 in 14% (10/73), 31 to 35 in 23% (17/73), 36 to 39 in 36% ( 26/73) and 40 to 45 in 23% (17/73) of cases (Figure 2). The gestational ages that amniocentesis was performed were: 15 weeks in 12% (9/73) of cases, 16 weeks in 32% (23/73), 17 weeks in 29% (21/73), 18 weeks in 12% (9/73), 19 weeks in 7% (5/73), 20 weeks in 3% (2/73), 21 weeks in 1% (1/73), 22 weeks in 3% (2/73) and 23 weeks in 1% (1/73).

In 92% (67/73) of cases one needle pass was needed, in 4% (3/73) two and in 4% (3/73) there were no details in the case notes (Figure 1). The time interval for results to be obtained was 0-9 days (1 day for 66% of cases) for FISH and 12-18 days for culture (14 days for 22%, 15 days for 40%, 16 days for 30% of cases). The chromosome results were normal in 93% (68/73) of cases, Down's syndrome in 4% (3/73) and Edwards in 3% (2/73). The maternal ages for Down's cases were 31, 32 and 38 years old. The outcome of pregnancies was: live births in 89% (65/73), stillbirths at 32 weeks and 35 weeks in 3% (2/73), miscarriages in 1% (1/73 at 19 weeks, 3 weeks after the amniocentesis), terminations in 7% (5/73, due to chromosomal abnormalities). Finally according to our records, 61 women delivered at term (84%) and 6 women (8%) delivered preterm. All results can be seen in Figures 3 and 4.




| MATERNAL AGE (YEARS) | NUMBER OF CASES |
|---|---|
| 20-25 | 3/73 **4%** |
| 26-30 | 10/73 **14%** |
| 31-35 | 17/73 **23%** |
| 36-39 | 26/73 **36%** |
| 40-45 | 17/73 **23%** |

**Figure 2.** Correlation between maternal age and number of women having amniocentesis.

| DAYS | FISH | DAYS | CULTURE |
|---|---|---|---|
| 0 DAYS | 1/73 **1%** | 12 DAYS | 1/73 **1%** |
| 1 DAY | 48/73 **66%** | 14 DAYS | 16/73 **22%** |
| 2 DAYS | 18/73 **25%** | 15 DAYS | 29/73 **40%** |
| 3 DAYS | 1/73 **1%** | 16 DAYS | 22/73 **30%** |
| 5 DAYS | 2/73 **3%** | 17 DAYS | 1/73 **1%** |
| 6 DAYS | 1/73 **1%** | 18 DAYS | 2/73 **3%** |
| 9 DAYS | 1/73 **1%** | | |

**Figure 3.** Speed of getting results following FISH and culture.

## Discussion

Amniocentesis remains the most common invasive prenatal diagnostic procedure today[3]. It is normally performed between 15 and 20 weeks of gestation. The use of amniocentesis prior to 14 weeks lead to higher pregnancy loss rates (7.6%), increase in talipes (1.3% as opposed to 0.1%) and an increase in amniotic fluid leakage (3.5% as opposed to 1.7%)[6]. The aim is to obtain fetal cells derived from skin, mucous membranes, amnion and umbilical cord for karyotyping or DNA analysis. It is performed under continuous ultrasound guidance by inserting a 22-gauze spinal needle transabdominally and with drawing a sample of amniotic fluid. The operator should try to avoid the placenta but it is more important to gain access to a deep, clear pool of liquor even if that means a transplacental approach. Fetal viability should always be checked before and after the procedure. Local anaesthesia is not necessary. Usually, 20 ml of amniotic fluid are enough. If the patient is Rh negative then 250 IU of anti-D immunoglobulin should be administered. The major risk of this procedure is that of miscarriage which is estimated between 0.5%-1%[7,8]. Other potential side effects include maternal infection, injuries and preterm labour[9]. That is the reason why amniocentesis is offered

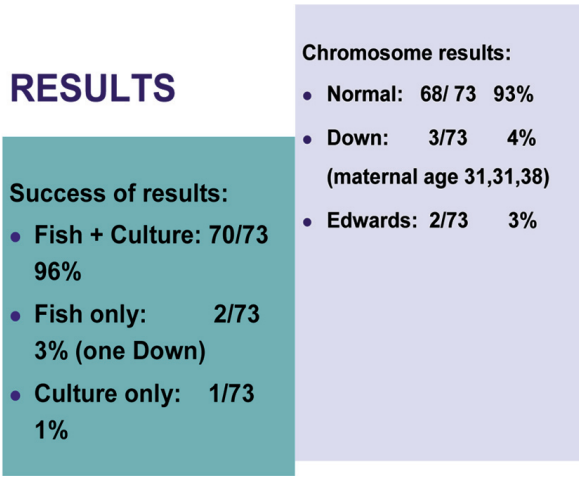



**Figure 4.** chromosome results following FISH and culture.

in high risk groups only. In order to determite the high risk group we used the first trimester screening test which combines maternal age, nuchal translucency measurement and biochemical maternal serum markers of b-hCG and PAPP-A. The detection rate for Down syndrome with this test is estimated to be about 90% and the false positive rate 5%[4,7,17].

The majority of chromosome abnormalities identified in prenatal samples are trisomy for chromosomes 13, 18, 21 and sex chromosome aneuploides. These are associated with the newborn phenotypes, Patau syndrome, Edwards syndrome and Down syndrome (trisomy 13, 18 and 21 respectively), and the less severe Turner (monosomy X) and Kleinnefelter (XXY) syndromes[10]. Down syndrome, with an incidence rate of 1 in 800 pregnancies, is the predominant reason for women seeking prenatal diagnosis. Karyotype analysis of cells by culture is usually available in more than two weeks times[8,11]. In order to reduce anxiety and improve pregnancy management, more rapid aneuploidy testing are used. The most widely established method is interphase-fluorescence in situ hybridization (FISH)[12-14]. Initially, a set of chromosome- specific fluorescence-labelled probes are hybridized to interphase nuclei of uncultured prenatal cells. The number of fluorescent signals in each nucleus obtained represents chromosome copy number. Usually 50 to 100 cells are analyzed to allow for low-level background and signal overlay that can occur during FISH procedures[15]. A quantitative fluorescence-PCR (QF-PCR) is a more recent addition to aneuploidy diagnosis[13,14]. The technique involves the relative quantification of microsatellite alleles to determine sequence copy number; amplification using fluorescence-labelled primers is followed by size separation and allele peak measurement on a semi-automated genetic analyzer. Results are usually available within 24 hours[16].

The results of our study come to agreement with international standards regarding interval for obtaining results[13,14] (1 day for 66% of cases with FISH and 12-18 days with culture). Also the complications rate (1% miscarriage rate) is acceptable[8,9]. It is essential that all centres undertaking prenatal diagnostic procedures carry out regular accurate audits of their results in order to ascertain the success and complication rates and to evaluate the service provided.

## References


1. Royal College of Obstetricians and Gynaecologists. Amniocentesis and chorionic villus sampling. Guideline No8. London: RCOG Press; 2005, [www.rcog.org.uk]
2. Mann K, Donaghue C, Fox SP, Docherty Z, Ogilvie CM. Strategies for the rapid prenatal diagnosis of chromosome aneuploidy. Eur J Hum Genet 2004; 12: 907-915
3. Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. Cochrane Database Syst Rev 2004; CD003252
4. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. Lancet 1998; 352: 343-346
5. Antsaklis A, Souka AP, Daskalakis G, Kavalakis Y, Michalas S. Second trimester amnicentesis vs chorionic villus sampling for prenatal diagnosis in multiple gestations. Ultrasound Obstet Gynecol 2002; 20: 476-481
6. Elias S, Simpson JL, Bombard AT. Amniocentesis and fetal blood sampling. In Milunsky A, ed. Genetic disorders and the fetus: diagnosis, prevention, and treatment. 4th ed. Baltimore: Johns Hopkins University Press 1998, pp 53-82
7. Benn PA. Advances in prenatal screening for Down syndrome: II first trimester testing,integrated testing, and future directions. Clin Chim Acta 2002; 324: 1-11
8. Bubb JA, Matthews AL. What's new in prenatal screening and diagnosis? Prim Care 2004 31: 561-82
9. The Canadian Early and Mid-trimester Amniocentesis Trial (CEMAT) Group. Randomised trial to assess safety and fetal outcome of early and midtrimester amniocentesis. Lancet 1998; 24351: 242-247
10. Daniilidis A, Klearhou N, Mauromichali M. Strategies for prenatal diagnosis today. Hippokratia 2006; 10: 22-27
11. Cameron A, Macara L, Brennand J, Milton P. Prenatal diagnostic techniques. In Fetal Medicine for the MRCOG and Beyond. RCOG Press 2002, pp 13-26
12. Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. Lancet 1986; 7: 1287-1293
13. Klinger K, Landes G, Shook D, et al. Rapid detection of chromosome aneuploidies in uncultured amniocytes by using fluorescence in situ hybridization (FISH). Am J Hum Genet 1992; 51: 55-65
14. Tepperberg J, Pettenati MJ, Rao PN, et al. Prenatal diagnosis using interphase fluorescence in situ hybridization (FISH): 2-year multi-center retrospective study and review of the literature. Prenat Diagn 2001; 21: 293-301
15. Thornton JG, Onwude JL. Prenatal diagnosis. In: Studd J,ed. Progress in Obstetrics and Gynaecology. London, Churchill Livingstone 1993; pp 13-31
16. Eiben B, Trawicki W, Hammans W, Goebel R, Pruggmayer M, Epplen JT. Rapid prenatal diagnosis of aneuploidies in uncultured amniocytes by fluorescence in situ hybridization. Evaluation of >3,000 cases. Fetal Diagn Ther 1999; 14: 193-197
17. Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years prospective experience. BJOG 2003; 110: 281-286