DonnishJournals

Donnish Journal of Medical Laboratory and Diagnosis
Vol 1(2) pp. 010-014 October, 2015.
http://www.donnishjournals.org/djmld

Original Research Article

Amniocentesis for Fetal Karyotyping during the third Quarter of Pregnancy. Indications and Results

Moez Kdous*, Oussema Khlifi, Marwen Braham, Jad Diari, Monia Ferchiou and Fethi Zhioua

Center of Reproductive Biology, Department of Gynecology and Obstetrics, Aziza Othmana Hospital, Tunis, University of Tunis El Manar, Tunisia.

Accepted 7th July, 2015.

Objective: **We aimed to evaluate the benefit of achieving a late amniocentesis in presence of one or more sonographic signs, in patients who are not at high risk for T21 at the first and second trimesters of pregnancy.** ***Methods:*** **Descriptive retrospective study conducted over 6 years from January 2008 through December 2013 in 222 pregnant women who have had amniocentesis for fetal karyotyping after 28 weeks of amenorrhea (WA).** ***Results:*** **306 amniocenteses for fetal karyotyping were analyzed. The mean age of the patients was 31 years (range: 17-45). Gestational age at amniocentesis was 32 WA (28-37 WA + 2 days). The mean interval between amniocentesis and delivery was 5 WA + 5 days. Nine (4.05%) cases of chromosome abnormalities were reported of which 6 (2.7%) were trisomy 21. The sonographic sign that generated the most amniocentesis was intrauterine growth restriction. The most correlated sign to trisomy was the atrioventricular canal defect. The two cases of polymalformative fetuses were related to trisomy 18. The karyotypes prepared in presence of a minor sonographic sign were all normal except for a partial trisomy 8 with dysmorphic corpus callosum on ultrasound.** ***Conclusion:*** **The third-trimester amniocentesis ensures the absence of chromosome abnormalities in patients initially classified at low risk during the first two trimesters of pregnancy, and in whom we discover late sonographic findings suggestive of aneuploidy. However, it must be applied only to specific situations and in countries where the legislation allows pregnancy medical termination of late stages. Although this practice has few complications, many of its indications should be reassessed.**

Keywords: Trisomy 21, Amniocentesis, Fetal malformation, Screening.

INTRODUCTION

Due to the development of screening techniques and early prenatal diagnosis of trisomy 21 in the first trimester of pregnancy, a process mandated by our society who is increasingly "in a hurry", the third-trimester amniocentesis for cytogenetic analysis has lost its interest over the years. However, practitioners are facing in some cases abnormalities diagnosable by ultrasound in the third trimester, with the possibility of a late amniocentesis to rule out any chromosome abnormalities before delivery.

This "rescue" amniocentesis is an alternative to the fetal blood sampling using puncture, which allows avoiding hospitalization, reducing fetal morbidity, for the price of a later outcome. This practice is getting increasingly scarce, and explains the few available studies on this subject. Also, it still poses some ethical problems: is it ethical to prepare a karyotype potentially followed by a termination of pregnancy in the third trimester? What are the indications that lead practitioners to propose these late amniocenteses? Are these indications really correlated to the risk of aneuploidy? The objective of this study was to evaluate the benefit of achieving a late amniocentesis in the presence of one or more sonographic signs, in patients who are not at high risk for trisomy 21 at the first and second trimesters of pregnancy.

MATERIALS AND METHODS

Data were collected retrospectively from birth registries, cytogenetic records, fetal sampling records and computer data

Corresponding Author: Moez Kdous. Center of Reproductive Biology, Department of Gynecology and Obstetrics, Aziza Othmana Hospital, Tunis, Medicine School of Tunis, University of Tunis El Manar, Tunisia. Tel: + 00216 21852683 Fax: + 0021671260174. E-mail: kdousmoez@gmail.com

from the obstetric and gynecological ultrasound data management software ASTRAIA® over a period of 6 years (January 2008-December 2013).

Population

a) Inclusion criteria

We have included in this study all amniocenteses made later in the third trimester of pregnancy (after 28 weeks of amenorrhea [WA]) in presence of at least one sonographic sign, previously unknown, or made later in patients who are not placed at risk for trisomy 21 during the first and second trimesters, even in case of incomplete screening. These sonographic signs have been described and validated for the detection of trisomy during the second trimester ultrasound [**1**]. We extrapolated these signs to use them in the third trimester, and classified them into 4 groups:

- Major signs: heart defects, complex fetus malformation, duodenal atresia.
- Minor signs: cerebral ventriculomegaly, short femur, bilateral pelvic dilatation, intestinal hyperechogenicity, agenesis of nasal bones, digestive duplication, renal dysplasia, abnormalities of the corpus callosum, clubfoot, and other minor signs.
- Severe intrauterine growth restriction (IUGR) (<3rd percentile).
- Isolated hydramnios.

b) Exclusion criteria

All amniocenteses made later in patients placed at risk for trisomy 21 during the first and second trimesters, and unwilling to take the risk of miscarriage were excluded. Also, were excluded all patients having amniocentesis with an intrauterine fetal death, patients who underwent amniocentesis in the context of viral (cytomegalovirus [CMV]) or parasitological (toxoplasmosis) seroconversion in addition to a systematic karyotype.

Study parameters

Study parameters were maternal age, gravidity, parity, nuchal translucency in mm, serum markers during the first trimester of pregnancy (free β-HCG (ng/ml) and PAPP-A (mU/l)), sonographic signs, gestational age at amniocentesis, karyotype results, pregnancy outcome (medical termination, intrauterine fetal death or live birth), term of delivery, and interval between amniocentesis and delivery.

Technique of amniocentesis

After sonographic identification of the placental position, a 20-gauge needle was inserted into the uterus and 20 to 30 ml of amniotic fluid were collected in strict asepsis and under ultrasound guidance. Amniocentesis was performed by different operators and patients could leave the hospital immediately after the end of the procedure. Corticosteroids for fetal maturation and tocolytics were not prescribed systematically. However, the patients were advised to rest for 24 hours and were informed about the signs that require an emergency department visit (uterine contractions, bleeding, rupture of the amniotic sac and signs of chorioamnionitis).

Chromosome analysis: FISH and culture

Chromosome analysis was performed in two stages: a fluorescent *in situ* hybridization (FISH) (with results within 24 to 48 hours) and cell culture (results in 15 to 21 days) for each sample and according the usual analytical techniques. Fifteen to 25 ml of amniotic fluid were cultured using Amniochrome II medium (Cambrex, Emerainville, France). All samples were analyzed in the same Cytogenetics Laboratory. Chromosomes were marked by bands using the Reverse Heat Giemsa technique (RHG).

Cytogenetic diagnosis was based on the analysis of chromosomes marked with RHG bands from at least 15 cell colonies grown *in situ*. AneuVysion® assay kit (VysisDowner's Grove, IL, USA) was used according to the manufacturers' instructions for identification of chromosome aneuploidies. This kit is composed of two probes, one for centromeric sequences for chromosomes X, Y and 18 and the other probe for loci on chromosomes 13 and 21.

Statistical analysis

Quantitative variables were summarized using median, 25th and 75th percentiles, and extreme values.

RESULTS

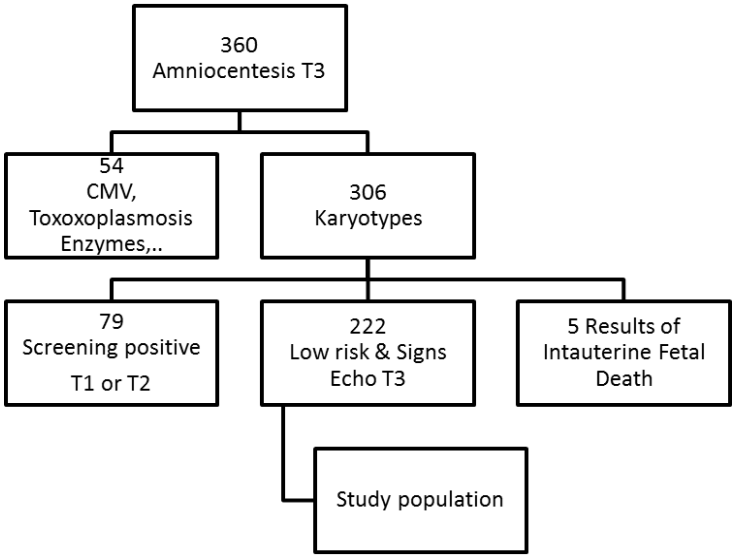
During the study period, we identified 360 samples that underwent amniocentesis after 28 WA. We excluded 54 for amniocentesis performed to detect infectious agents (CMV, toxoplasmosis) or metabolic assays (enzyme research,...). Over the 306 amniocenteses for fetal karyotyping, 222 (72.5%) were performed following late sonographic findings in patients initially classified at low risk of trisomy 21 or who were not screened. Five patients underwent amniocentesis to assess an unexplained intrauterine fetal death (IUFD). The remaining patients were classified at risk during the first or second trimesters of pregnancy, but preferred to undergo a third-trimester amniocentesis to replace a risk of miscarriage by a risk of premature delivery.

Figure 1 displays the distribution of these patients. The mean age was 31 ± 5.3 years[range: 17-45 years], and11.4% of the patients were aged over 38 years old. Mean gravidity was 2.3 ± 1.2, and 16.7% of the women were at their first pregnancy. A combined screening during the first trimester was carried out in 82.4% of the cases, and the mean gestational age at amniocentesis was 32 ± 2.2 WA [range: 28-37 WA + 2 days (Table 1).

The main sonographic findings that advised the performance of amniocentesis are detailed in Table 2. Nine (4.05%) cases of chromosome abnormalities were reported, with trisomy 21 being the most frequent abnormality (6 [2.7%] cases). Karyotypes with minor deficiencies were not reported because they were considered as normal variants.

As regards the sonographic findings that implicated the preparation of karyotypes and the pregnancy outcome, the mean delivery term was 37 WA + 6 days ± 2 WA[range: 29-42 WA]. The mean interval between amniocentesis and delivery was 5 WA + 5 days. We reported a preterm labor followed by a birth in the week following sampling in 12 cases (5.4%), of which 2 (0.9%) correspond to the ruptures of membranes with one proven chorioamnionitis. We also reported 3 cases of fetal deaths due to fetal pathology, namely one case of hypoplastic left heart with significant heart failure, and two cases of severe IUGR with abnormal fetal Doppler (Table 3).

Figure 1: Study population



Abbreviations: CMV=cytomegalovirus, T=trisomy.

Table 1: Main characteristics of women at inclusion

	Mean± Standard deviation N (%)
Maternal age (years)	31 ± 5.3
Gravidity	2.3 ± 1.2
Parity	1.1±1.0
Interval between amniocentesis and delivery (weeks of amenorrhea)	32 ± 2.2
First-trimester screening	187 (84.2%)
Nuchal translucency (mm)	1.4 ± 0.5
PAPP-A (mU/l)	3258 ± 2049.3
Free β-HCG (ng/ml)	43.7 ± 38.1

Abbreviations: β-HCG= beta human chorionic gonadotropin; PAPP-A=pregnancy-associated plasma protein A.

Table 2: Distribution of the main indications of amniocentesis by sonographic signs

Indication of amniocentesis	N	%	Trisomy
Major signs	21	9.4	6
AVCD	4	1.8	3
Membranous VSD	4	1.8	1
Other heart defects	11	5.0	0
Duodenal atresia	0	0.0	0
Malformation syndrome	2	0.8	2
Minor signs	129	58.1	1
Cerebral ventriculomegaly	19	8.6	0
Short long bones	13	5.9	0
Bilateral pelvic dilatation	12	5.4	0
Intestinal hyperechogenicity	8	3.6	0
Clubfoot	7	3.2	0
Abnormalities of the corpus callosum	6	2.7	1
Agenesis of nasal bones	5	2.3	0
Digestive duplication	5	2.3	0
Renal dysplasia	4	1.8	0
Other	50	22.3	0
IUGR	49	22.1	2
Hydramnios	23	10.4	0

Abbreviations: AVCD= atrioventricular canal defect; IUGR=intrauterine growth restriction; VSD=ventricular septal defects

Table3: Details of diagnosed cases of trisomy

Age (years)	First-trimester screening	Sonographic signs	Interval between amniocentesis and delivery (WA)	Trisomy	Outcome	Delivery term (WA)
45	Unwanted	Late severe IUGR	32	21	Medical termination	35
33	NT=2.2	Malformation syndrome	33	18	Medical termination	35
26	CR 1/1300	Isolated AVCD	35	21	Medical termination	37
34	NT=1.3	Isolated AVCD	33	21	Live birth - operated	38
31	RC 1/4955	Thick corpus callosum	34	Partial 8	Live birth – mental retardation	36
40	No – late discovery	AVCD + Short femur	34	21	Medical termination	35
43	CR 1/680	Malformation syndrome	32	18	Intrauterine fetal death	36
37	CR 1/320	Membranous VSD	32	21	Medical termination	34
31	CR 1/1460	Late severe IUGR	31	21	Medical termination	33

Abbreviations: AVCD=atrioventricular canal defect; CR=combined risk; IUGR=intrauterine growth restriction; NT=nuchal translucency; VSD=ventricular septal defects; WA=week of amenorrhea.

DISCUSSION

We remind that 19 to 36% of trisomic fetuses show no detectable morphological abnormalities on ultrasound [**2**] However, chromosome abnormalities may have phenotypic expression detectable on ultrasound during the second and third trimesters of pregnancy.

While the literature is rich in papers about sonographic signs suggestive of aneuploidy in the second trimester, publications on the third-trimester ultrasound are scarce [**3**]. Therefore, practitioners tend to extrapolate the signs of the second trimester to prepare a fetal karyotype. Benacerraf [**1**] was the first to distinguish between the major and minor sonographic signs on the second-trimester ultrasound. The recognition of a major malformation is likely to directly indicate a fetal karyotype. In parallel, the interpretation of the minor signs requires some correlation with other risk factors, such as maternal age, obstetric history, and first-trimester screening [**4**].

The prenatal prevalence of major signs is 46 to 65% [**5**]. Congenital heart disease is a very good sign of chromosome abnormality, and is the most common morphological abnormality in trisomy 21, affecting nearly 50% of fetuses. In particular, the atrioventricular canal defect (AVCD) is the main heart condition, representing 75% of heart diseases diagnosed in trisomy 21. In case of congenital heart disease, the current risk of trisomy is 10%, all-heart diseasesconfused [**6**]. These data are consistent with our results, since the AVCD was the most frequently sign encountered (75%). Four of the 19 fetuses (21.05%) analyzed for congenital heart disease had trisomy 21. The duodenal atresia was also found in 7 to 8% of trisomy 21, with the prevalence of trisomy in case of isolated duodenal atresia is approximately 30% [**7**]. In our study, none of the fetuses had this abnormal condition.

As regards the minor signs, their list underwent frequent changes over the years. Their use in the third trimester is more difficult, due to the entanglement of several superimposed parameters. As a result, some of these signs are detected (meaning a short femur detected at the third trimester? Meaning of pyelic hypotonia of 5 mm?...). In a low-risk population, the identification of minor signs has been very insensitive because almost all karyotypes made following these signs were normal [**8**].

Also, abnormalities of the central nervous system have a variable diagnostic interest. Ventriculomegaly and hydrocephalus allow the screening for 0.5 to 5% of trisomy. Furthermore, the choroid plexus cysts are associated with trisomy 21 in less than 1% of cases, when the kysts persist during the third trimester [**9**]. As regards the ~~face~~ facial abnormalities, they are controversial for their low specificity. In fact, the diagnosis of facial dysmorphia is very difficult with low specificity even with the use of 3D ultrasound; only an associated malformation calls for a screening for trisomy 21. All uropathies are chromosome abnormality with an overall sensitivity of approximately 12%. However, pyelectasis is more specific for trisomy 21, it is pathological when the anteroposterior diameter of the pelvis is greater than 5 mm between 20 and 30 WA and greater than 7 mm after 30 WA. Pyelectasis can be unilateral or bilateral, and its sensitivity is low in low-risk population when pyelectasis is isolated [**9**].

Regarding biometric signs, femur length is the reference parameter for the late detection of aneuploidies, particularly trisomy 21. Since the femur is significantly shorter (<3rd percentile) during the post-natal screening in 60% of children with trisomy 21, several publications have studied this sign in the pre-natal phase. We also highlight that the diagnosis of short femur has a low sensitivity on ultrasound ranging between 19 and 38%, with a false positive rate of 4.7 to 8.8% [**10**]. However, in presence of one or two associated anomalies, the prevalence of trisomy 21 reaches 15 and 45% respectively.

In a meta-analysis conducted by Odibo *et al.* [**10**] in January 2014, the minor signs that predicted most aneuploidy in the second trimester were moderate ventriculomegaly, increase of the thickness of the neck soft tissues, intestinal hyperechogenicity and absence or hypoplasia of the nasal bones.Verdin *et al.* reported a significantly increased risk of aneuploidy in the subgroup of women at low risk of trisomy, in case only one minor sonographic sign was present [**11**]. In our study, no case of trisomy was diagnosed following the detection of one of the minor signs cited by Odibo. However, only one case of partial trisomy 8 was identified in a fetus who had a thick and short corpus callosum. The difference between the literature and our results could be related to the ultrasound's timing. Indeed, Verdin reported these signs in the second trimester [**11**], while in our study, ultrasound was performed at 32 WA, when the interpretation of the same signs may be different.

The risk of aneuploidy also increases with the number of anomalies and defects. Nicolaides *et al.* reported a higher risk

for aneuploidy in the context of multiple malformation syndromes (29%) in comparison with the fetus having a single defect (2%) [**12**]. This is consistent with our results where the only two malformation syndromes have been associated with trisomy 18. These two patients were classified in a low-risk population during the first-trimester screening.

The late severe intrauterine growth restriction (<3rd percentile) is rarely isolated, and is often associated with fetal pathology or malformation, even if the karyotype was normal. Therefore, the identification of a severe IUGR during the third trimester justifies the preparation of a fetal karyotype even when the first-trimester screening was low risk [**13**]. By cons, when IUGR is rarely moderate (3^{rd} to 10^{th}percentile). In our study, IUGR was the most common indication for the performance of late amniocenteses (22.1%). Of the 49 fetuses analyzed for IUGR, only 2 (4.08 %) had trisomy.

Concernig the pregnancy outcome after amniocentesis, the identification of late amniocentesis complications was poorly evaluated in the literature with only six published studies and one having the karyotyping as indication [**14**]. Gordon *et al* reported complications in 0.7% of the collected samples [**15**], *versus* 0.9% in our study. In contrast, Hodor *et al.* conducted a case-control comparative study and found no increase in the number of complications related to the gesture during the first 48 hours [**16**].

CONCLUSION

The third-trimester amniocentesis ensures the absence of chromosome abnormalities in patients initially classified at low risk during the first two trimesters of pregnancy, and in whom we discover late sonographic findings suggestive of aneuploidy. However, it must only be applied to specific situations and in countries where the legislation allows pregnancy medical termination of late stages. Although this practice has few complications, many of its indications should be reassessed, as evidenced by our results. Finally, would not the main indication for the third-trimester amniocentesis be simply the anxiety of the physicians themselves?

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Benacerraf BR, Frigoletto FD, Laboda LA. Sonographic diagnosis of Down syndrome in the second trimester. Am J Obstet Gynecol 1985,153:49–52.
2. Boog G. Screening for chromosomal abnormalities using ultrasound. In: Institut national de la santé et de la recherche médicale, Dommergues M, Aymé S, Janiaud P, Seror V, ed. Diagnotic prénatal. Pratiques et enjeux. Paris: Inserm; 2003. p. 168–209.
3. Drummond CJ, Molina Gomes D, Senat MV et al. Fetal karyotyping after 28 weeks of gestation for late ultrasound findings in a low risk population, Prenat Diagn 2003, 23: 1068–1072.
4. Malone FD, Canick JA, Ball RH, et al. First-trimester or second trimester screening, or both, for Down's syndrome. N Engl J Med 2005,353:2001–11.
5. Stoll C, Alembik Y, Dott B, Roth MP. Study of Down syndrome in 238 942 consecutive births. Ann Genet 1998, 41:44–51.
6. Paladini D, Tartaglione A, Agangi A, et al. The association between congenital heart disease and Down syndrome in prenatal life. Ultrasound Obstet Gynecol 2000,15:104–108.
7. Breathnach FM, Fleming A, Malone FD. The second trimester genetic sonogram. Am J Med Genet Part C Semin Med Genet 2007, 145:62–72.
8. Rozenberg P, Malagrida L, Cuckle H, et al. Down's syndrome screening with nuchal translucency at 12–14 weeks and maternal serum markers at 14–17 weeks: a prospective study. Hum Reprod 2000, 17: 1093–1098.
9. Rotmensch S, Liberari M, Bronshtein M, et al. Prenatal sonographic findings in 187 fetuses with Down syndrome. Prenat Diagn 1997, 17: 1001–1009.
10. Odibo AO, Ghidini A. Role of the second-trimester 'genetic sonogram' for Down syndrome screen in the era of first-trimester screening and noninvasive prenatal testing. Prenat Diagn 2014,10:1002,4329.
11. Verdin SM, Whitlow BJ, Lazanakis M, Kadir RA, Chatzipapas I, Economides DL. Ultrasonographic markers for chromosomal abnormalities in women with negative nuchal translucency and second trimester maternal serum biochemistry. Ultrasound Obstet Gynecol 2000, 16: 402–406.
12. Nicolaides KH, Snijders RJM, Cosden RJM, Berry C, Campbell S. Ultrasonographically detectable markers of fetal chromosomal abnormalities. Lancet 1992, 340: 704–707.
13. Vintzileos AM, Guzman ER, Smulian JC, Mclean DA, Ananth CV. Choice of second-trimester genetic sonogram for detection of trisomy 21. Obstet Gynecol 1997, 90: 187–190.
14. Picone O, Fuchs F, Sénat MV et al. [Evaluation of the third trimester amniocentesis for fetal karyotyping in women with fear of pregnancy loss]. J Gynecol Obstet Biol Reprod (Paris). 2008, 37(4):385–391.
15. Gordon MC, Narula K, O'Shaughnessy R, Barth Jr WH. Complications of third-trimester amniocentesis using continuous ultrasound guidance. ObstetGynecol 2002,99:255–259.
16. Hodor JG, Poggi SH, Spong CY, Goodwin KM, Vink JS, Pezzullo JC, et al. Risk of third-trimester amniocentesis: a case-control study. Am J Perinatol 2006, 23:177–180.