J of Obst. & Gyn. of India Vol. 51, No. 5 · September/October 2001 Pg 114-117

Aneuploidy Screening on Uncultured Amniocytes in Selected Referral Groups – A Clinical Audit.

Nandita Maitra, P.J. Simpson, J.J. Waters, E.Roberts, E.V. Davison

Fetal Medicine Unit, Birmingham Women's Hospital and Regional Cytogenetics Centre, Birmingham Women's Hospital, Birmingham.

Summary

We present a clinical audit of aneuploidy screening on uncultured amniocytes from two referral groups: abnormal ultrasound scan and Down syndrome risk (low AFP) > 1:100. Ninety five samples were screened, 68 samples were received for an abnormal ultrasound scan and 27 for increased Down syndrome risk. Fluorescent in situ Hybridization (FISH) screening detected 17 unequivocal aneuploidies. The same referral group revealed 26 abnormalities detected by conventional cytogenetic analysis. All 20 aneuploidies were unequivocally confirmed by conventional cytogenetics together with six structural abnormalities not detected by FISH. This clinical audit with a limited data set, highlights the fact that selection of a high-risk referral group that has a relatively high risk of FISH detectable aneuploidy, co-selects for structural chromosome abnormalities which subsequently require rapid, high quality cytogenetic analysis.

Introduction

We present a clinical audit of aneuploidy screening on uncultured amniocytes from two referral groups

1. Abnormal ultrasound scan
2. Advanced maternal age and/or maternal serum screen double test (MSSDT) risk > 1:100 at consultant request.

Ninety-five samples received between 01/01/98 and 21/05/99 were screened. Sixty-eight samples were referred because of an abnormal scan and 27 for consultant request because of increased Downs syndrome risk defined by serum screening. Tests were performed using commercial probe sets LSI 13/21 and CEP X/Y/18 (Vysis).

We reviewed: 1) the cytogenetic findings by gestational age and reason for referral 2) the FISH assay results and 3) the abnormalities present in the sample group. We investigate the outcome of pregnancies in which an abnormality was detected and finally we correlate FISH and cytogenetic amniotic fluid analysis findings.

This clinical audit on a limited data set seeks to evaluate the use-effectiveness of the FISH technology versus conventional karyotyping in a high risk referral group.

Clinical Protocol

All amniotic fluid specimens referred for rapid detection of aneuploidies by FISH were simultlaneously processed for cytogenetic analysis. A 2-3 ml aliquot was

used for FISH and was processed to permit two independent hybridizations. Probe sets for chromosomes 13/21 and 18/X/Y were used depending on the request received from the referring physicians. Clumped nuclei, nuclei with attached cytoplasm or cellular membrane and nuclei which looked similar to polymorphonuclear cells were not scored. Each case was analysed by two independent scorers. A conclusive test has been defined as a minimum of 50 cells scored with 25 cells checked, atleast 65 percent of which must show a consistent signal pattern.

Results

Table I shows that most of the amniocenteses (84.2%) were performed between 16 and 24 weeks gestational age. Eight (8.4%) tests were performed before 16 weeks in which 3 abnormalities were detected: one fetus with nuchal translucency >6mm had Trisomy 21, two fetuses with cystic hygromata had Monosomy X. Eight (8.4%) tests were performed after 24 weeks of which one fetus with abnormal growth profile was found to have Trisomy 18 at 35 weeks. Three aneuploidies were detected in this group.

Table II shows the number of FISH assays. Nine cases were selected for FISH screening from amniotic fluids received between 01.01.98 to 2 and represent 1.78% of this work load. Mean rep time for all FISH assays was 4 days. Cytogenetic a on these samples was completed within the ran 22 days.

Table III shows the correlation between res amniocentesis, karyotype and FISH assays. O cases with a normal FISH result, cytogenetic a revealed 46 normal karyotypes, six str abnormalities, one Trisomy 21 (which was not c as only X/Y/18 probe was applied) and one fa the 17 cases reported as being abnormal on F were confirmed on karyotype. Four samples in th although strictly inconclusive (12-37 nuclei score reported as abnormal on FISH (>50% of nuclei Trisomy 21). FISH failed in four cases, three c had a normal karyotype and one of which was 18. Of the 20 cases reported as FISH inconcl had a normal karyotype, one was 45X and o cytogenetically. No cases reported as abnorma were subsequently shown to have normal kary

Table I
Cytogenetic analysis by weeks of gestation

Gestation	12/13	14/15	16/17	18-20	21-24	25-28	29-32
Trisomy 21 (08)	-	1	4	2	1	-	-
a) MSSDT	2						
b) abscan	1	2	2	1			
Trisomy 18	-	-	2	1	2	-	-
b) abscan	2	1	2	1			
Trisomy 13	-	-	1	2	1	-	-
b) abscan	1	2	1				
Monosomy X	1	1	-	-	-	-	
b) abscan	1	1					
Structural chr.anom.	-	-	1(bal.RTL)	1	4	-	-
b) abscan	-	-	1	4	-	-	-
Normal karyo (69)	2	3	12	27	19	1	3
a) MSSDT (24)	1	1	10	10	1	1	-
b) abscan (45)	1	1	11	13	14	-	3
Total number referred (95)	3	5	20	33	27	1	3

Table II
Numbers of FISH assays

	Cases	Tests
FISH performed	95	166
Samples tested with LSI 13/21 and CEP 18/X/Y	71	142
No. tested with 13/21 or 18/X/Y only	24	24

Table III
Correlation between results of amniocentesis Karyotype and FISH assays

FISH result Amnio result	FISH Normal	FISH Abnormal	FISH INCN	FISH FAIL	Total
Normal	46	0	18	3	67
Abnormal	7	17	1 (45X)	1 (T18)	26
Fail	1	0	1	0	2
Total	54	17	20	4	95

Incn: inconclusive (<50 cells available to score)

With reference to the abnormal karyotypes detected (Table IV) of the six fetuses with Trisomy 21 and abscan, 3 had pleural/pericardial effusions with increased nuchal translucency. All the six fetuses with trisomy 18 had abnormalities on scan. There were five fetuses with structural chromosomal abnormalities and abnormal scan findings – diaphragmatic hernia [del (15) and der (13) t (4;13), omphalocoele and ventriculomegaly (add 9p), cardiac defect der (5) t(5:8) and choroid plexus cyst inv 95)]. Both fetuses with Monosomy X had cystic hygroma. The der(13)t (4;13) was suspected to be a cultural artefact and cord blood sampling after birth revealed a normal karyotype.

Twenty of the 26 women opted to have termination of pregnancy (TOP) (Table V). Of the 18 women who had both an abnormal karyotype and anomalies on ultrasound, 12 underwent TOP after the final cytogenetic report, 3 had TOP on the basis of ultrasound findngs and before the final cytogenetic results. One with a Trisomy 18 fetus and two with Trisomy 21 fetuses opted to continue pregnancy and all three conditions were confirmed on cord blood samples after birth. In all women that opted to continue pregnancy the diagnosis of aneuploidy had been made prior to 22 weeks gestation. Of the five fetuses with structural chromosomal abnormalities, two had TOP on ultrasound findings alone, [der (5) with cardiac defect and del (15) with diaphragmatic hernia] and one after the cytogenetic report.

Discussion

This is a clinical audit of aneupolidy screening on uncultured amniocytes using fluorescent insitu hybridization in two high risk referral groups. FISH has been successfully performed on amniocytes from 12 to 36 weeks gestation. Tests were performed using

Table IV
Abnormal Karyotypes detected

Anomaly detected	Cytogenetics	FISH
Trisomy 21	8	7+(01 not detected, only CEP X/Y/ 18 requested)
Trisomy 13	4	4
Trisomy 18	6	5 + (1 fail)
Monosomy X	2	1+(1 inconclusive)
Structural chromosomal anomalies	6	Not detectable with probes used
Total	26	17

Table V
Outcome of pregnancy by Karyotype and scan findings

Karyotype and scan findings	TOP prior to cytogenetic report	TOP after cytogenetic report	Continuation of pregnancy
MSSDT risk with abnormal karyotype and normal scan	-	2	-
Abscan with aneuploidy	3	12	4
Structural chromosomal anomalies with abscan	2	1	2

commercially available probe sets LSI 13/21 (Vysis) and CEP X/Y/18 (Vysis). Fluorescent insitu Hybridization (FISH) screening detected 17 unequivocal aneuploidies. One trisomy 18 was not detected due to technical failure and one 45, X karyotypic outcome was inconclusive with FISH. One trisomy 21 was not detected because a trisomy 18 only screen was requested. The same referral group revealed 26 abnormalities detected by conventional cytogenetic analysis. All 20 aneuploidies were unequivocally confirmed by conventional cytogenetics together with six structural abnormalities not detected by FISH. All cases were reported within 22 days (mean 12.6), whereas mean FISH reporting time was 4 days. Five women opted for termination, on the basis of scan and FISH reports, 15 opted to do so after the final cytogenetic report, six women with abnormal scan findings continued with their pregnancies. This group consisted of T-18, T-21, rob (14;21) and der 13(t) (4;13). The abnormal scan referral group accounted for 5/6 structural abnormals and 18/20 FISH detected aneuploidies. On the basis of this audit we propose the following conclusions.

- Selection of high risk referral groups for FISH detectable aneuploidy (abscan and MSSDT) co-selects for structural chromosome abnormalities which subsequently require rapid high quality cytogenetic analysis.

- FISH will rapidly and accurately detect 13,18,21 and X and Y aneuploidies but will fail to detect most structural abnormalities. (Eiben et al, 1998; Spathas et al, 1994).

Reporting of provisional results on FISH analysis should stress that a full karyotype has still to be performed and that the FISH result excludes trisomy (for the chromosomes tested) only.

Potential disadvantages of this technology include increased maternal anxiety following an uniformative result and the negative effect of receiving a disomic FISH result followed by the identification of a chromosomal lesion not identified by the FISH protocol. (Ward et al 1993).

Acknowledgement

This audit was performed when the first author was on a fellowship at the Fetal Medicine Unit, Birmingham Women's Hospital. The authors are grateful to Professor Martin J. Whittle for the use of the data presented in this audit.

References

1. Eiben B, Trawicki W, Hammans W, Goebel R, Epplen JT. Prenatal Diagnosis. Vol 18(9): page 901, 1998.
2. Spathas DH, Divane A, Maniatis GM, Ferguson-Smieth ME, Ferguson-Smith MA. Prenatal Diagnosis, Vol 14(11); page1049, 1998.
3. Ward BE, Gersen SL, Carelli MP, McGuire NM, Dackowski WR, Weinstein M, Sandlin C, Warren R, Klinger KW, Am. J. Hum. Genet. Vol 52: page 854, 1993.