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Amniocentesis in the West Midlands: report on 1000 births

TESSA WEBB,* J H EDWARDS,* A H CAMERON,†
J MARGARET CRAWLEY,‡ MAJ HULTEN,§ D I RUSHTON,‖
AND R A THOMPSON¶

*From the *Departments of Clinical Genetics, ‡Biochemistry, and ‖Pathology, Birmingham Maternity Hospital; †Department of Pathology, Birmingham Children's Hospital; and the §Regional Cytogenetics Laboratory, and ¶Department of Immunology, East Birmingham Hospital, Birmingham*

SUMMARY Two laboratories in the West Midlands have monitored 1000 'at risk' pregnancies. Of these 57% were referred for chromosomal indications and 43% for possible neural tube defects. The largest at risk groups (both 37%) were those mothers who had already had a pregnancy resulting in a baby with a neural tube defect (21% spina bifida and 16% anencephaly), and those who were referred because of the increased risk of Down's syndrome in pregnancies where the mother was over 35 years old. Six percent had already borne a child with Down's syndrome.

An estimate of the AFP level in the amniotic fluid was achieved in 985 (98·5%) of the pregnancies. Of these, 967 mothers could be reassured that the baby did not have an open neural tube defect and 18 abnormal fetuses were terminated or died spontaneously.

Chromosome studies were completed in 846 (85%) of the pregnancies with the consequence that 19 were terminated. Of these, 15 had an abnormal karyotype, including nine with Down's syndrome. Four male fetuses were terminated because of a high chance of carrying X linked disease.

The 1000 pregnancies monitored required 1080 amniocenteses. Cases were excluded from this study if the amniocentesis took place after the 20th week of gestation and if follow-up data concerning the outcome had not been received. This follow-up data was obtained through a questionnaire, but only ten cases were lost to the study at this point, mainly because the subject had moved away from the area before the birth of her child.

This report describes the amniocentesis service offered by the West Midlands Regional Health Authority. The area serves a population of $5{\cdot}5 \times 10^6$ and there are about 65 000 births a year. The first 200 pregnancies monitored were part of a pilot study carried out in the Department of Cancer Studies of the University of Birmingham between 1972 and 1975 and are not included in this report.

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In the Autumn of 1975 the diagnostic amniocentesis service in the West Midlands was established in two areas, one served by the laboratories at Birmingham Maternity Hospital (BMH) and the other by those at East Birmingham Hospital (EBH). Since then 1000 pregnancies of known outcome have been monitored. The distribution of these between the two laboratories is shown in table 1.

TABLE 1 *Total number of pregnancies monitored*

Laboratory	*Total No of amniocenteses performed*	*Total No of amniocentesis failures*	*Total No of births*	*Males*	*Females*	*No of twin births*
BMH	531	46	474	216	196	3
EBH	549	6	526	475	434	4

	No of spontaneous abortions	*No of stillbirths or NND*	*No of terminations*
BMH	10	9 + (1)*	13 + (3)†
EBH	17	7	18 + (4)†

*Meckel's syndrome with 2 failed amniocenteses.
†Includes terminations of male fetuses at risk for X linked disease.

Indications for amniocentesis

The distribution of all the cases by indication is given in table 2. An expanded version giving miscellaneous indications in more detail is given in table 3.

It can be seen that, unlike some other studies,[1 2] the numbers presented for chromosomal and neural indications are approximately equal.

DOUBLE INDICATIONS

In all, 64 pregnancies had double indications. In each case the primary indication used for this study was the one defined by the consultant obstetrician (table 4).

TABLE 2 *Distribution of cases by indication*

Chromosomal indications	*No*
Maternal age 40+	243
Maternal age 35–39	125
Previous child with trisomy 21	63
Previous child with other aneuploidies	8
Previous child with other chromosomal abnormalities	6
Parental translocation	7
Family history of trisomy 21 (not previous child)	49
Previous congenital abnormalities in child	31
X linked disease	11
Others	19
Total	562
Neural tube defect and related indications	*No*
Previous child with spina bifida	199
Previous child with anencephaly	158
Previous child with exomphalos	1
Previous child with Finnish nephrotic syndrome	1
Previous child with hydrocephaly	22
Family history of NTD (excluding previous child)	39
Previous children with neural tube defect (two)	5
Neural tube defect in parent	9
Other	4
Total	438

TABLE 3 *Expansion of indications*

	No
Chromosomal	
Previous child with trisomy 18	4
Previous child with trisomy 13	1
Previous child with triploidy	1
Previous child with XYY syndrome	1
Previous child with Turner's syndrome	1
Total	8
Previous intersex child	1
Drug therapy in parent	7
Anxiety over age (less than 35 years)	6
X-rays of mother during present pregnancy	2
Previous spontaneous abortion	2
Threatened abortion in present pregnancy	1
Total	19
Neural tube defect	
Unusual ultrasound of present pregnancy	3
Hydramnios of present pregnancy	1
Total	4
X linked disease (mother suspected carrier)	
Muscular dystrophy	5
Haemophilia	4
Immunodeficiency	1
Retinitis pigmentosa	1
Total	11

In 37 cases one indication was chromosomal and the other for a neural tube defect. Of 27 mothers over 35 years old who had already had a neural tube defect pregnancy, six aborted or were terminated or had a stillbirth.

HIGH α-FETOPROTEIN PREGNANCIES

In all, 17 abnormal pregnancies were detected; 16 of these were terminated and one case of Meckel's syndrome died after birth. During this pregnancy amniocentesis failed twice, but the serum AFP level was very high.

The 17 abnormal pregnancies could be divided into nine anencephalics, five with spina bifida, one with Finnish nephrotic syndrome, one macerated fetus, and one with Meckel's syndrome.

Five of these pregnancies had been referred for amniocentesis with chromosomal indications, four for maternal age, and one who had already borne a child with Down's syndrome.

CHROMOSOMAL ABNORMALITIES

All chromosomal abnormalities detected are listed in table 5 together with the outcome of the pregnancy and the age of the mother. Of the 20 chromosomal anomalies detected (2·0%), nine were of

TABLE 4 *Pregnancies with double indications*

Laboratory	*No in which one indication was chromosomal and one NTD*	*No in which both indications were chromosomal*	*Others*
BMH	19	6	10
EBH	18	6	5

TABLE 5 *Chromosomal abnormalities detected*

Karyotype	*Outcome*	*Maternal age*
46,XX,22p+	TNF	30
47,XY,+G	TDO	33
47,XY,+G	TDO	41
47,XX,+G	TDO	45
47,XY,+G	TDO	45
46,X,+frag	LTU	40
47,XY,+D	TPA	37
46,XX/47,XX,+2	NF	40
47,XY,+G	TDO	44
47,XX,+E	TED	41
46,XX/45,XO or 45,X	NF	25
46,XX,t(6;7)	NF	29
46,XY,t(6;10)	NM	22
47,XXY	TKL	44
48,XXYY	TXXYY	26
47,XX,+21	TDO	46
47,XY,+21	TDO	38
46,XY,t(2;9)	TM	39
47,XX,+21	TDO	44
47,XX,+21	TDO	45

20 abnormal karyotypes, 15 terminations, 4 normal livebirths, 1 abnormal livebirth.
LTU, live Turner's syndrome; TNF, terminated normal female; TDO, terminated trisomy 21; TPA terminated trisomy D; TED terminated trisomy E; TKL, terminated XXY; NF, normal female; NM, normal male; TM, terminated male.

Down's syndrome (0·9%) resulting in each case in a termination. Other terminations included one with trisomy 18, one with trisomy 13, one with XXY, one with XXYY, and one de novo balanced translocation. Two balanced translocations and two apparent mosaics came to term with the birth of apparently normal babies. One fluid was diagnosed as 46,XX,22p+; the pregnancy was terminated and the fetus appeared to be a normal female. One pregnancy with a 46,X,+fragment came to term (parental decision) with the birth of a female baby with signs consistent with Turner's syndrome.

Two of the balanced translocations and the fetus with the 48,XXYY karyotype were referred for amniocentesis with neural tube defect indications.

Four fetuses with a 46,XY normal male karyotype were aborted because of a high risk of carrying X linked disease.

GESTATION AT AMNIOCENTESIS

The ideal time for amniocentesis to be performed is at 16 weeks of gestation. Before this gestation, the volume of amniotic fluid may be low and α-fetoprotein levels are less discriminatory. Beyond 16 weeks, the time for all the analyses before the possible abortion of an abnormal fetus becomes critical. Table 6 gives the distribution of gestation for the first amniocentesis in the 1000 cases studied. The percentage of culture failures decreases with increasing gestation but the rate of spontaneous fetal loss is independent of gestational age at amniocentesis.

Diagnostic results obtained

It was not possible to combine the diagnostic results obtained from the two laboratories because, although dry taps were regularly reported by the Birmingham Maternity Hospital laboratory (where 47% of the specimens were obtained on site), at East Birmingham Hospital (which is some miles from the nearest obstetric unit), no amniocentesis failures were reported, and only six specimens were unsuitable. A check at one particular hospital which keeps very efficient records showed that amniocentesis failures were not always being reported to the laboratory, although they were being recorded.

For this reason, the diagnostic results obtained are not comparable and have been compiled separately. For a true picture of the success rate all dry taps should be reported.

PREGNANCIES WITH NEURAL TUBE DEFECTS

Those pregnancies which had raised α-fetoprotein levels because of a neural tube defect or, in one case, the Finnish nephrotic syndrome, are shown in table 7. One high AFP level has been attributed to intrauterine death and is not included in the table. The level of discrimination achieved is shown in fig 1 and 2. In these figures, the logarithm of the α-fetoprotein value is plotted against the gestation in weeks for each pregnancy.

Table 8 presents a summary of chromosome success rate related to the success of the amniocentesis itself, and shows that blood stained samples

TABLE 7 *Raised α-fetoprotein pregnancies*

α-fetoprotein level (μg/ml)	*Gestation*	*Outcome*	*Maternal age*
182	16	TSB	31
Serum (955 μg/l)	18	DMK	23
265	16	TAN	32
525	16	TAN	26
411	17	TFS	32
420	15	TAN	44
77	16	TSB	27
340	16	TAN	47
41	15	TSB	26
320	19	TAN	36
390	16	TAN	31
190	20	TAN	25
400	17	TM*	39
93	16	TSB	24
91	15	TSB	40
395	17	TAN	29
401	15	TAN	32
Total = 17 pregnancies (1·7%)			

TSB, terminated spina bifida; TAN, terminated anencephalic; TFS, terminated Finnish syndrome; TM*, terminated macerated fetus; DMK, neonatal death Meckel's syndrome.

TABLE 6 *Gestation at first amniocentesis*

Gestation	*No*	*No of spontaneous abortions*	*No of stillbirths or NND*	*No of culture failures*			
				BMH	*EBH*	*Total*	*%*
12	2	—	—	—	—	—	—
13	8	1	—	1	—	1	(12·5)
14	63	2	2	8	5	13	(21)
15	155	3	3	17	15	32	(21)
16	403	12	7	64	17	81	(20)
17	165	5	2	15	8	23	(14)
18	119	0	1	9	5	14	(12)
19	54	1	1	7	3	10	(19)
20	31	1	—	3	1	4	(13)
Total	1000			124	54	178	

are, surprisingly, not necessarily more likely to fail. The degree of contamination by red blood cells was a subjective observation by the laboratory staff and need not be comparable between the two laboratories. More chromosome failures occurred in samples which had been obtained without the aid of ultrasound.

An increase in the level of spontaneous fetal loss was observed in the group where the chromosome had failed and also when the amniocentesis itself

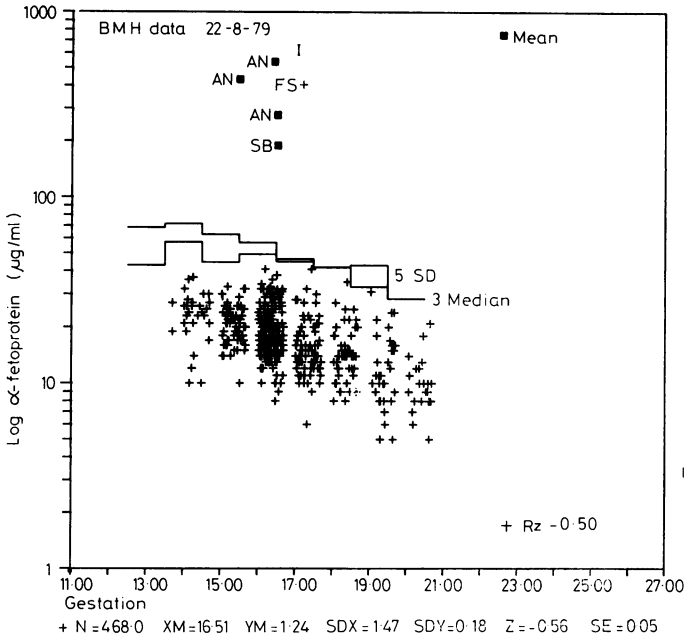

FIG 1 *Log α-fetoprotein against gestation for pregnancies monitored at Birmingham Maternity Hospital. Discriminatory lines are at 5 times standard deviation and 3 times median. +, births with normal outcome; AN, anencephalic; SB, spina bifida; FS, Finnish nephrotic syndrome; I, intrauterine death.*

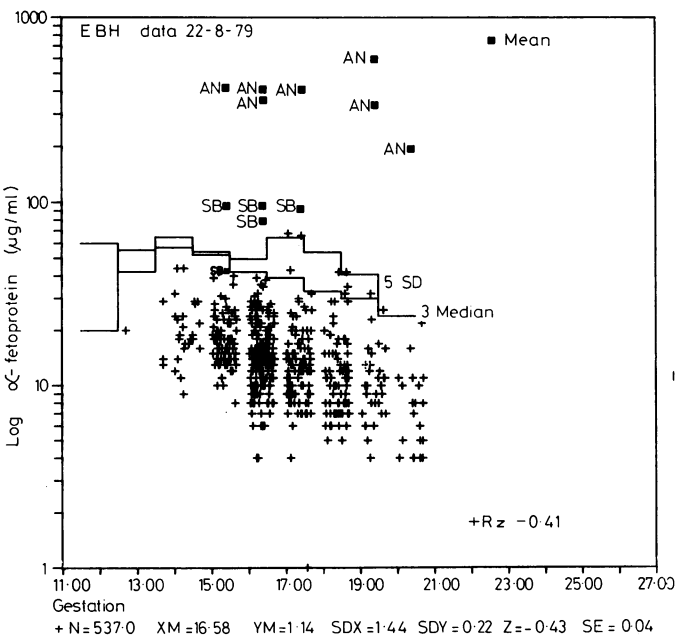

FIG 2 *Log α-fetoprotein against gestation for pregnancies monitored at East Birmingham Hospital. Discriminatory lines are at 5 times standard deviation and 3 times median. +, births with normal outcome; AN, anencephalic; SB, spina bifida.*

TABLE 8 *Chromosome success rate related to success of amniocentesis*

	Blood stained samples			
	No of samples with chromosome results	*No of samples with chromosome failure*	*Amniocentesis failures*	
BMH	9 (60%)	6	3	
EBH	26 (93%)	2	—	
	Samples obtained without ultrasound			
	Chromosomes normal, outcome normal	*Chromosomes fail, outcome normal*	*Chromosomes normal or abnormal, outcome abnormal*	*Amniocentesis failure*	*Total*
BMH	21 (52·5%)	15 (37·5%)	1 (2·5%)	3 (7·5%)	40
EBH	46 (85%)	4 (7%)	2 (4%)	2 (4%)	54

Chromosome failures (of first amniocentesis fluid sample received)

	Total	*Spontaneous abnormal outcome*	*Terminations*
BMH	124	7 (6%)	3
EBH	54	6 (11%)	1

Amniocentesis failures

	Total	*Spontaneous abnormal outcome*
BMH	46	5 (+ one infected placenta)

TABLE 9 *Summary of fetal losses or abnormalities*

Laboratory	*Total loss*	*Abnormal livebirth*	*Abortion or IUD*	*Stillbirth or NND*	*Termination*	*NTD death*	*Total births*
EBH detected	18				18		
EBH normal fetus loss	28		17	7	4		526
BMH detected	15	1			13	1	
BMH normal fetus loss	22		10	9	3		474
Total normal fetus loss	50		27	16	7		1000
%	5·0		2·7	1·6	0·7		
Normal fetus loss in advanced age	18		12	4	2		420*
%	4·3		2·9	0·95	0·5		

*Includes those with double indications

had failed. When the amniocentesis had to be repeated, there were three cases of spontaneous fetal loss from 57 repeated cases.

Discussion

High α-fetoprotein levels were detected in the amniotic fluid of 16 pregnancies, resulting in each case in the termination of an abnormal fetus. The cut-off point for normality was taken at 3 × median, but the 5 × SD would discriminate similarly (fig 1, 2). Lines representing both types of cut-off are shown in the figures. One of the laboratories estimated several values lying within the cut-off or 'grey' area including one from a spina bifida fetus which was later terminated. Although several of the fluids were heavily blood stained, the fluids from fetuses with neural tube defects were also blood stained. Some of the patients were referred to Professor S Campbell for spinal scanning. No normal fetuses were later terminated.

The smaller number of amniocentesis failures was reported from the half of the region containing the greater number of small hospitals. In the western half of the region, fluids were received from six centres, three of which sent fewer than 50 samples. By contrast, the laboratories in the eastern half of the region served 14 centres, ten of which sent fewer than 50 fluid samples. The fetal loss rates reflected in these figures were 19/474 in the western region and 24/526 in the other (table 9). In the former, if an amniocentesis 'kit' is broached, then the number is recorded even if the amniocentesis itself fails. In neither centre, however, was the number of needle insertions recorded as has been recommended.

Chromosome studies were attempted on all fluids received despite the pressure on laboratory staff and facilities. One of the reasons for chromosome failure was that insufficient fluid was obtained. The proportion of samples referred for chromosomal indications has risen from 45% in 1975 to 65% in 1978, while the absolute numbers have more than doubled themselves each year. The level of chromosome failure has fallen throughout the three years taken to complete this study. It is felt that laboratories offering this service should not become overstretched too quickly.

Samples from outlying hospitals reached the laboratories by first class mail but the delay very rarely amounted to more than one day and did not seem to influence the viability of fluid cells. The success rates achieved by the chromosome laboratories can be calculated with respect to either patient numbers or to the number of samples actually received. The BMH laboratory had a 75% overall success rate with respect to the numbers of patients, while a chromosome result was obtained on 74% of the samples received. At EBH a successful chromosome culture ensued from 90% of the samples received, while the success rate per patient reached 93%.

No chromosome abnormalities were missed and, in the 40+ age group, 11 abnormalities were detected in 243 pregnancies (4·5%), whereas in the 35 to 39 age group, three abnormalities were detected in 125 pregnancies (2·4%).

Of the 16 pregnancies with neural tube defects, ten were referred because of a previous NTD, giving a detection rate of 10/362 (2·8%).

Very few cases were encountered in which there was no apparent clinical reason for amniocentesis (table 3). The case in which the indication given was a threatened abortion in the present pregnancy did in fact result in a spontaneous abortion four days after the amniocentesis.

Although there were 38 terminations, 31 were as a consequence of a detected abnormality and four were of male fetuses with a high risk of carrying disease. Of the remaining three, one woman had a large ovarian cyst, one apparently normal pregnancy was terminated after the finding of a 46,XX, 22p+ karyotype (this woman has subsequently had a perfectly normal pregnancy), and one woman had a private social termination of her pregnancy. Her indication for amniocentesis was for her age of 39 years.

Two of the balanced translocations were de novo, and one set of parents elected for a termination.

Both pregnancies with mosaic chromosome results were allowed to continue with the birth of two normal female babies. The extra number 2 chromosome was present in only one colony, while a repeat amniocentesis of the apparent Turner's syndrome pregnancy yielded a sample with only 46,XX cells. The pregnancy ended with the birth of a normal female infant, exactly as has been reported in a previous case.[3]

Altogether 918/1000 pregnancies resulted in a live baby (91·8%). No chromosome result was obtained in 154 of these. Of the 43 spontaneous fetal losses, 13 had a chromosome failure. So of 154 failures, 13 fetuses were spontaneously lost (8·4%). This is considerably more than the 50/1000 (5%) fetal loss overall.

There were seven errors in predicting the sex of the fetus, all from one laboratory (BMH). Four were diagnosed as 46,XX and a male was born, and three had the less likely combination of a 46,XY chromosome result being followed by the birth of a female infant.

Throughout this study, the age of the mothers to whom amniocentesis was offered because of the increased risk of Down's syndrome was 38 years, although younger women were accepted at the discretion of the obstetricians. During 1976, however, only 4·6 pregnancies in 1000 livebirths were tested, whereas in other regions up to 32·6 pregnancies in 1000 livebirths received amniocentesis.[4] A survey within the region has shown that patients at certain centres are at an advantage even within the West Midlands Region itself.

In an attempt to estimate the risks in the procedure, the numbers of fetal losses after amniocentesis were compared with those suffered by all patients in one large hospital (BMH). Although this is not a true control group, only fetal loss after 16 weeks of gestation was considered, and the group was divided into mothers above and below the age of 35 to eliminate the age bias inherent in the subjects. Amniocentesis carried a risk of 1 to 1·5% to the fetus, which is in agreement with other findings.[5]

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Requests for reprints to Dr Tessa Webb, Department of Clinical Genetics, Birmingham Maternity Hospital, Birmingham B15 2TG.