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Prenatal detection of trisomy 21: combined experience of two British hospitals

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A retrospective study was performed to determine the detection rate of trisomy 21 in two British hospitals using a combination of: (1) second trimester serum screening with maternal age, αFP and hCG; (2) karyotyping for raised maternal age and high background risk of aneuploidy; and (3) second trimester fetal anomaly ultrasonography at 18–22 week gestation. 36 410 women with a median age of 27 years were studied. Trisomy 21 detected by the combination of methods in both hospitals was compared with the actual number of pregnancies affected by trisomy 21, to determine the detection rate. Serum screening as the backbone of the service detected 31/48 (65%) trisomy 21 affected pregnancies. Karyotyping for maternal age and previous aneuploidy detected eight trisomy 21 affected pregnancies, and second trimester ultrasound a further six, giving a total detection rate of 45/56 (80%). Thus, the detection rate of trisomy 21 in our population is 65% by serum screening alone. This is similar to demonstration projects, but the addition of second trimester ultrasonography and karyotyping for maternal age and prior risk, contributes further to improve the overall sensitivity to 80%. The invasive procedure rate was 4.8% of all women.

KEY WORDS: trisomy 21; serum screening; second trimester ultrasound; karyotyping for maternal age and prior risk

INTRODUCTION

Age related screening has largely been replaced by screening based on second trimester biochemical markers in Britain. (Wald *et al.*, 1992; Haddow *et al.*, 1992). Various combinations of alpha-fetoprotein (αFP), human chorionic gonadotrophin (hCG), free β-human chorionic gonadotrophin (free βhCG) and unconjugated oestriol (uE_3) are utilized, the most popular being the double or triple test. (Wald *et al.*, 1992; Haddow *et al.*, 1992; Macri *et al.*, 1994) Almost all women in Britain are additionally offered and undergo fetal ultrasound examination at 18–22 weeks' gestation. Major abnormalities related to trisomy 21 may be detected during this examination, and in recent years a number of so-called minor ultrasound markers, which may impart a variable risk of chromosomal abnormality, have been described, e.g. short femur, fetal pyelectasis, echogenic bowel, mild ventriculomegaly and nuchal skin thickness. (Benacerraf *et al.*, 1987; Bromley *et al.*, 1994; Vintzileos and Egan, 1995; Nicolaides *et al.*, 1992b; Nyberg *et al.*, 1990). Furthermore, there are women either with sufficient risk or sufficient anxiety who directly request karyotyping and bypass screening systems. As a result, the detection of trisomy 21 has become a 'package' where all three techniques are available to all women in many maternity units in Britain. (Smith and Hau, 1999). Data on actual detection rates being achieved in clinical practice using these three methods is, however, scarce.

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Two units in our Region commenced second trimester serum screening with maternal serum αFP and hCG (double test) in 1992. Karyotyping for maternal age or high background risk of aneuploidy was available and all women were offered fetal ultrasound examination at 18–22 weeks' gestation. The aim of this study was to determine, for audit purposes, the number of trisomy 21 affected pregnancies detected by second trimester serum screening and primary karyotyping in our population and what impact second trimester ultrasound diagnosis had on overall detection rates.

METHODS

This retrospective study was carried out at the Countess of Chester Hospital (COCH), a District General Hospital, and the Liverpool Womens Hospital (LWH), a University teaching hospital. The time period studied was February 1992 to January 1997 for COCH and August 1992 to July 1995 for LWH.

Gestational age determination

Gestational age was calculated from last menstrual period for those with regular cycles and from ultrasound measurement of the crown–rump length (Robinson and Fleming, 1975) in those with irregular periods, pill periods or those who were unsure of their dates at COCH. Gestational age was calculated from ultrasound measurement of the crown–rump length (Robinson and Fleming, 1975) at LWH.

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Serum screening

All women booking before 20 weeks' gestation were offered serum screening with αFP and hCG between 15 and 20 weeks' gestation, after counselling by the booking midwife. Serum αFP and hCG concentrations were measured in multiples of median adjusted for maternal weight, by fluorometric immunoassay using Amerlex-M 2T kits (Ortho Clinical Diagnostics, Amersham, UK). Batch size was not fixed. Inter-batch imprecision for αFP revealed a coefficient of variation (CV) of 4.5%, 3.1% and 4.3% at mean concentrations of 11, 32 and 115 μg/l respectively and hCG, 5.3%, 3.3% and 3.4% at concentrations of 9.4, 30 and 176 mU/l respectively. The mean bias for αFP, relative to all-laboratory trimmed mean, has run over the past six months at +12.8% and for hCG at −0.5%. Karyotyping was offered at a risk cut-off of 1 : 250. A specialist midwife counselled women in both populations in the event of an abnormal test. The age distribution of the screened population was the age at the time of the test. The age related risk of a positive test (risk 1 : 250 or above) in each age distribution group was calculated as a ratio of the total number of women in that age group having a serum screen.

Karyotyping for maternal age and prior risk

Women over the age of 39, those with a previous aneuploid pregnancy or those referred from primary care were offered the choice of amniocentesis at 15 weeks' gestation, first trimester chorionic villus sampling (CVS) or serum screening with αFP and hCG between 15 and 20 weeks' gestation.

Second trimester fetal anatomy scan

A fetal anatomy ultrasound scan for the detection of fetal abnormality and chromosomal markers at 18–20 weeks gestation, was carried out by trained obstetric sonographers. This was open to all women. The protocol for these scans included measurement of biparietal diameter, abdominal circumference and femur length (Chitty *et al.*, 1994a,b,c) as well as assessment of fetal lie, amniotic fluid and placental site. Attempts were made to visualize lateral ventricles, choroid plexus, cerebellum, cisterna magna, cavum septum pellucidum, longitudinal and transverse view of the spine, anterior abdominal wall including cord insertion, four-chamber view of the fetal heart and atrio-ventricular (AV) valves, kidneys, stomach, bladder, extremities, face, hands, feet and gender. Pregnancies with major malformations associated with trisomy 21 (e.g. AV canal defects and duodenal atresia) or minor markers were referred for ultrasound examination by obstetricians or radiologists trained in fetal medicine or obstetric ultrasound. The minor markers were: fetal pyelectasis measuring >5 mm in the antero-posterior diameter (Benacerraf *et al.*, 1990), hyperechogenic bowel as bright as bone (Bromley *et al.*, 1994; Nyberg *et al.*, 1990), shortened humerus/femur length (Benacerraf *et al.*, 1987, 1991), mild ventriculomegaly characterized by an atrial diameter of the lateral ventricle measuring 10–12 mm (Bromley *et al.*, 1990) and increased nuchal skin thickness in the midsagittal view producing a tremor on balottement/>5 mm at the occiput (Nicolaides *et al.*, 1992a; Benacerraf *et al.*, 1985). Following this further examination, the women were counselled by the sonologist on the risk of chromosomal abnormality using currently available data (Benacerraf *et al.*, 1987; Vintzielos *et al.*, 1995; Nicolaides *et al.*, 1992b) and karyotyping was offered. All karyotyping procedures were performed under real time ultrasound guidance by or under direct supervision of practitioners trained in obstetric ultrasound. Both hospitals employed the services of the Merseyside and Cheshire Regional Cytogenetics Laboratory.

Result retrieval

Records of all raised serum screening results (1 : 250 or above), between February 1992 and January 1997 for COCH and between August 1992 and July 1995 for LWH, were obtained from the Departments of Chemical Pathology of the respective hospitals. Risk estimates and age were noted and the outcome of these records analysed in terms of uptake of karyotyping, result of karyotype, and normality at birth. Karyotype procedures performed between these dates for maternal age, anxiety, previous aneuploidy, as well as for ultrasound abnormalities, were identified from records kept by the specialist midwife, of all karyotyping procedures at COCH and from the Regional Cytogenetics Laboratory at LWH. The results were analysed and the outcome of pregnancies obtained. Women with a raised serum screen risk were eliminated from the ultrasound group even if an ultrasound anomaly was subsequently detected. Pregnancies with trisomy 21 which were not detected antenatally but diagnosed postnatally were identified from the cytogenetics laboratory. These had not been detected antenatally because of false negative screening, maternal refusal of testing or refusal of karyotyping following a raised serum screen risk.

RESULTS

Of 36 410 women offered maternal serum screening in the study period, 26 080 (72%) chose to have the test. 370 women (1%) had karyotyping by request, 9960 (27%) declined serum screening or screening by maternal age/prior risk. All these women had a second trimester ultrasound for fetal anatomy.

Antenatal detection of trisomy 21 (Figure 1)

Serum screening

There were 1354 positive tests out of 26 080 serum screens (5.2% screen positive rate). Following counsel-

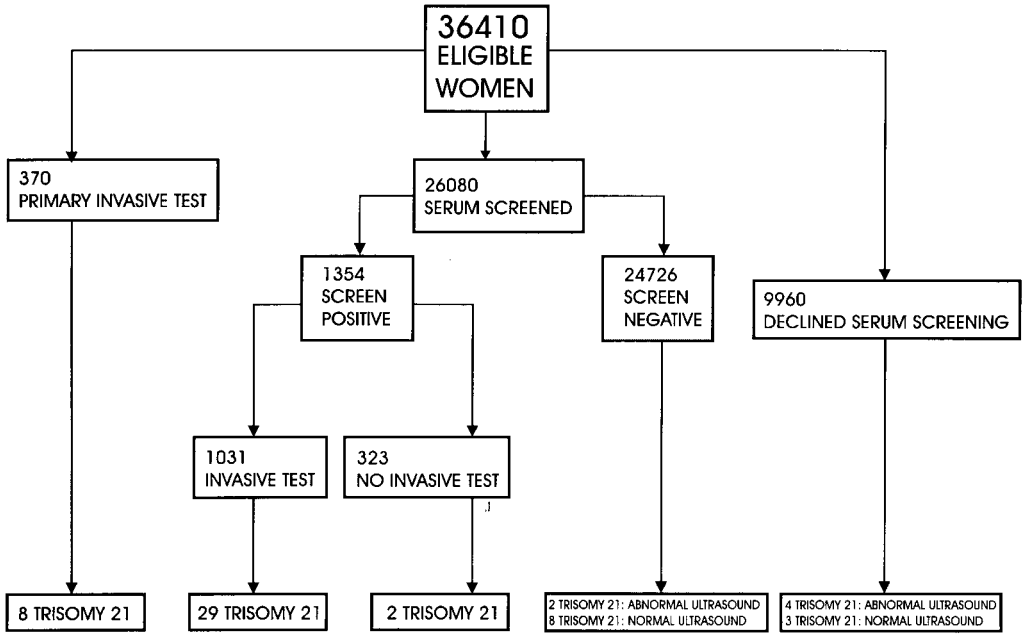

Figure 1—Antenatal detection of trisomy 21 (Countess of Chester and Liverpool Women's Hospitals 1992–1997)

ling by a specialist midwife, 1031 (76%) chose an invasive test and 323 declined further testing. There were 31 fetuses with trisomy 21 in this group but two were not confirmed antenatally because the mothers declined karyotyping despite a positive screen.

There were 31 true positives, 1323 false positives, 24 716 true negatives and 10 false negatives in the serum screened population of 26 080. The false positive rate was 5% (1323/[26 080–41]) The sensitivity of serum screening alone is 76% and the specificity 95%. The positive predictive value of a serum screen in our population is 2.3% and the negative predictive value 99.96%.

The mean age was 27 years (range 14–55). The age related risk of a positive serum screen in our population is as shown in Table 1. The risk of having a pregnancy affected by trisomy 21 in the serum screened group is shown in Table 2.

Karyotyping for maternal age and prior risk

370 women chose to undergo diagnostic testing by karyotyping, without prior serum screening on the basis of their age/anxiety/background risk. Eight fetuses with trisomy 21 were identified in this group.

Second trimester ultrasound

Second trimester ultrasound was performed on the remaining 24 726 women with a low risk serum screen

Table 1—Age related risk of a positive serum screen in the study population

Age group	<20	20–24	25–29	30–34	35–36	37–38	39–40	41–42	43–44	45+
Total screened	1533	4973	8737	7993	1552	902	401	127	25	9
Number positive	34	125	288	493	207	208	146	56	13	8
Risk of positive test	1:45	1:40	1:30	1:16	1:7	1:4	1:3	1:2	1:2	1:1

Table 2—Age related risk of having an affected pregnancy in the serum screened population

Age group	<25	25–29	30–34	35–36	37–38	39+
Total screened	6506	8737	7993	1552	902	562
True positives	4	4	10	5	5	3
False negatives	2	2	3	2	0	1
Risk of having an affected pregnancy	1:1084	1:1456	1:615	1:222	1:180	1:140

Table 3—Details of trisomy 21 pregnancies detected by second trimester ultrasound

Maternal age	Ultrasound abnormality	Serum screen
34	Fetal pyelectasis	1 : 770
25	Ventricular septal defect	1 : 5000
32	Cardiac abnormality	no screen
28	Dandy–Walker syndrome	no screen
36	4 × choroid plexus cysts	no screen
34	Cystic hygroma and bilateral short femurs	no screen

and on the 9960 women who declined or were too late for serum screening. Karyotyping was performed on 359 (1%) women for abnormal ultrasound scans. We do not know how many women with abnormal ultrasound declined invasive testing and therefore do not know the total number of abnormal ultrasound scans. Six fetuses with trisomy 21 were identified by ultrasound, two had a low risk serum screen and four were in pregnancies where the mother had declined serum screening (Table 3).

Summary of results: performance of total package

Serum screening as the backbone of the service detected the most trisomy 21 affected pregnancies. Karyotyping for maternal age/high risk of aneuploidy detected 14% of trisomy 21 affected pregnancies. Second trimester ultrasound contributed to the detection of a further 11%, identifying two of the 10 false negatives in the serum screen group and four of seven trisomy 21 pregnancies in the group that declined serum screening. The combination of the three methods ultimately missed 11 trisomy 21 pregnancies, – eight had negative serum screen and no abnormality on ultrasound, three had no serum screening and normal ultrasound.

Serum screening and invasive testing on the basis of maternal age detected 69% of trisomy 21 affected pregnancies in our population. The addition of second trimester ultrasound improved the overall detection rate to 45/56 (80%) for a 4.8% invasive procedure rate.

Procedure details

The miscarriage rate for amniocentesis was 0.6% and ~1.5% for first trimester chorionic villus sampling. There were no fetal losses from placental biopsy or fetal blood sampling.

DISCUSSION

The 80% overall detection rate of trisomy 21 in our population, reflects what was achieved using all available modalities with serum screening as the backbone of the service. 4.8% of the population required an invasive karyotype procedure to achieve this. The invasive procedure rate required in our population to achieve a high detection rate for trisomy 21 is similar to that quoted for first trimester screening programmes using nuchal translucency (Snijders *et al.*, 1998).

Serum screening replaced a policy of screening on the basis of age alone in our population, with amniocentesis for maternal age still open to women above the age of 39. The odds of having a positive test in this age group are 1 in 3, and the odds of having an affected pregnancy in the event of a positive test is 1 in 140. It is difficult to draw many conclusions from these results because 370 women chose to forgo serum screening for a diagnostic test, but if all women above the age of 39 had chosen a diagnostic test, the overall invasive procedure rate would have been much higher at 6.4%. It would appear appropriate to continue to offer karyotyping on the basis of maternal age, but the option of maternal serum screening can be retained for those who prefer a less invasive method.

Serum screening detected the most trisomy 21 affected pregnancies in our study. The uptake rate of 72%, although similar to that of triple test demonstration projects (Wald *et al.*, 1992, 1998), was lower than the 88% mean uptake rate quoted in double test demonstration projects (Wald *et al.*, 1998). Our lower uptake rate probably reflects the population studied. The uptake of a diagnostic test in the event of a raised risk was 76% and this compares favourably with previous published figures (Wald *et al.*, 1992; Dawson *et al.*, 1993; Haddow and Palomaki, 1996; Lam *et al.*, 1998). Given the high uptake rate of a diagnostic test in the event of a raised risk, it may be possible to further improve detection rates by improving the overall uptake of serum screening through counselling and education (Marteau, 1992).

The 1 in 644 prevalence of Down syndrome in the study population, and 1 in 44 prevalence in the raised serum screen group, is similar to other published results for screening using the double (Dawson *et al.*, 1993) and triple test (Haddow *et al.*, 1992; Wald *et al.*, 1992). In the lower risk serum screen group, however, the risk is much lower than published figures, at 1 in 2472 (Haddow *et al.*, 1992; Wald *et al.*, 1992). The detection rate of trisomy 21 in women offered serum screening was 31/48 (65%). This detection rate is comparable to double test demonstration projects (Spencer and Carpenter, 1993; Macri *et al.*, 1994) and only 6% lower than the observed mean detection rate of 70% for triple test demonstration projects (Wald *et al.*, 1998). If only those who accepted serum screening were considered, as in demonstration projects, the detection would be far higher at 31/41 (76%).

Eight further cases of trisomy 21 were detected in women who chose to have a diagnostic test, a prevalence of 1 in 46. This suggests that it is reasonable to allow women who have selected themselves as 'high risk' on clinical grounds, to continue to have access to diagnostic procedures without the need for other screening. The 11% contribution that second trimester ultrasound makes to the overall detection of trisomy

21, in those pregnancies deemed low risk by maternal serum screening and in those in whom no prior screening has been performed, is an important finding of this study. Four of seven trisomy 21 pregnancies in women who declined serum screening were detected by ultrasound alone. Ultrasound has been shown to identify around 6–9% of trisomy 21 affected pregnancies when used as a screening method in a low risk population (Chitty *et al.*, 1991; Goncalves *et al.*, 1994). Our results compare favourably with this. Detection rates using a single second trimester marker are too low for ultrasound to be used as a primary screening method but it is of value in improving the overall detection rate. Recently, Smith and Hau (1999) showed a 20% improvement in detection of all trisomy 21 if detailed scanning at 18–22 weeks was undertaken. The findings from our study would certainly support this.

This study was retrospective in design because we were interested in determining how well the package of three modalities performed in terms of detecting trisomy 21. The study did not address the issues of maternal anxiety from false positives or the cost implications of our programme, although we agree that these are important considerations in any health package. It has demonstrated, however, that using the three methods available, a practice common to many hospitals in the UK, 80% of trisomy 21 pregnancies can be detected. The approach outlined here provides women with choices ranging from declining serum screening to requesting diagnostic karyotype procedures directly. We have also demonstrated that routine ultrasound provides a clinically useful improvement in detection of trisomy 21 in a population with a high uptake of serum screening.

Many issues need to be considered when determining service provision for detection of trisomy 21. These include dissemination of information to women, detection rates, procedure rates, timing of testing, cost of the service and how it interacts with existing established screening. In our practice, high detection rates are achieved with a system which offers women time to make choices and allows women wishing primary karyotyping to opt in, whilst missing out only a few women who present late. Amniocentesis was the invasive procedure used most and this has the advantage of detecting, by the safest method, affected pregnancies which are destined to continue. The introduction of any new screening methods in our region must provide either distinct advantages to the women, improvement in detection rate or reduction in cost without reduction in detection rate.

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