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Screening for trisomy 21 by fetal tricuspid regurgitation, nuchal translucency and maternal serum free β-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks

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KEYWORDS: free β-hCG; nuchal translucency; PAPP-A; prenatal screening; tricuspid regurgitation; trisomy 21

ABSTRACT

Objective *To examine whether in pregnancies with fetal trisomy 21 the level of maternal serum free ß-human chorionic gonadotropin (ß-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 to 13 + 6 weeks' gestation is independent of the presence or absence of tricuspid regurgitation and to estimate the performance of a screening test that combines tricuspid regurgitation with fetal nuchal translucency (NT) thickness and serum free ß-hCG and PAPP-A.*

Methods *The study population comprised 77 trisomy 21 and 232 chromosomally normal fetuses from singleton pregnancies at 11 + 0 to 13 + 6 weeks of gestation. In all cases the fetal karyotype was determined by chorionic villus sampling (CVS), which was carried out at the request of the parents after first-trimester screening for trisomy 21 by fetal NT and maternal serum free ß-hCG and PAPP-A. Immediately before chorionic villus sampling, fetal echocardiography was performed and the presence or absence of tricuspid regurgitation was determined by pulsed wave Doppler ultrasonography. The distribution of fetal NT, maternal serum free ß-hCG and PAPP-A in trisomy 21 fetuses with absent and present tricuspid regurgitation was examined. We examined two screening strategies: first, integrated first-trimester screening in all patients and second, first-stage screening of all patients using fetal NT and maternal serum free ß-hCG and PAPP-A followed by second-stage assessment of tricuspid regurgitation only in those with an intermediate risk of 1 in 101 to 1 in 1000 after the first stage.*

Results *Tricuspid regurgitation was observed in 57 (74.0%) of the trisomy 21 fetuses and in 16 (6.9%) of the chromosomally normal fetuses. There were no significant differences in median maternal age, median gestational age, free ß-hCG multiples of the median (MoM) and PAPP-A MoM in trisomy 21 fetuses with and without tricuspid regurgitation. The modeled detection rates of trisomy 21 for fixed false positive rates of 1%, 2% and 5% in screening by maternal age, fetal NT thickness and maternal serum free ß-hCG and PAPP-A and assessment of tricuspid flow in all cases were 87%, 90% and 95%. In the two-stage approach, the estimated detection rate was 91% and the false positive rate was 2.6%.*

Conclusions *There is no relationship between tricuspid regurgitation and the levels of maternal serum free ß-hCG and PAPP-A in cases with trisomy 21. An integrated sonographic and biochemical test at 11 + 0 to 13 + 6 weeks can potentially identify about 90% of trisomy 21 fetuses for a false-positive rate of 2–3%. Copyright © 2005 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Effective screening for trisomy 21 is provided in the first trimester of pregnancy by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 to 13 + 6 weeks of gestation. It has been shown prospectively that, for a false positive rate of 5%, the detection rate of trisomy 21 by this method is about 90%, which is superior to the 30% achieved by maternal age alone or the 65% by maternal age and second-trimester serum biochemistry[1].

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A new first-trimester sonographic marker of trisomy 21 is tricuspid regurgitation, determined by pulsed wave Doppler ultrasonography[2]. A study of fetuses at 11 + 0 to 13 + 6 weeks' gestation reported the presence of tricuspid regurgitation in 8.5% of 458 chromosomally normal fetuses and in 65.1% of 126 with trisomy 21[3]. Furthermore, the study demonstrated that in both the normal and the trisomic fetuses, the prevalence of tricuspid regurgitation decreases with fetal crown–rump length (CRL) and increases with NT thickness, and established likelihood ratios for trisomy 21 in fetuses with and without tricuspid regurgitation at the 11 + 0 to 13 + 6 weeks' scan.

The aim of this study was to examine whether the level of maternal serum biochemical markers was independent of the presence or absence of tricuspid regurgitation and to estimate the performance of a screening test that integrates the two sonographic and two biochemical markers. We examined two screening strategies: first, integrated first-trimester screening in all patients and second, first-stage screening of all patients using fetal NT and maternal serum free β-hCG and PAPP-A followed by second-stage assessment of tricuspid regurgitation only in those with an intermediate risk of 1 in 101 to 1 in 1000 after the first stage[1].

METHODS

The study population comprised 77 trisomy 21 and 232 chromosomally normal fetuses from singleton pregnancies at 11 + 0 to 13 + 6 weeks of gestation. In all cases the fetal karyotype was determined by chorionic villus sampling, which was carried out at the request of the parents after first-trimester screening for trisomy 21 by fetal NT and maternal serum free β-hCG and PAPP-A, at the Fetal Medicine Centre, London[1,4].

The maternal serum free β-hCG and PAPP-A were measured using the Kryptor analyzer (Brahms AG, Berlin, Germany), and an ultrasound examination was carried out to measure the fetal NT and CRL and to diagnose any major defects. All scans were carried out by sonographers who had obtained The Fetal Medicine Foundation Certificate of Competence in the 11 + 0 to 13 + 6 weeks' scan (www.fetalmedicine.com). Patient specific risks were calculated by a multivariate approach using population parameters established in our retrospective study, and the maternal age and gestation related risk of trisomy 21 at the time of screening[5,6]. Essentially, the maternal age-related risk was multiplied by each likelihood ratio (LR) derived from the fetal NT and maternal weight-adjusted serum free β-hCG and PAPP-A. The maximum and minimum LRs allowed were 0.12 and 55 for NT, 0.018 and 7.138 for each metabolite and 0.1 and 80 for the combined sonographic and biochemical markers.

Immediately before chorionic villus sampling, fetal echocardiography was performed transabdominally and the presence or absence of tricuspid regurgitation was determined by pulsed wave Doppler ultrasonography[3]. The sample volume was placed across the tricuspid valve in an apical four-chamber view and it was positioned so that the angle to the direction of flow was less than 30°. Tricuspid regurgitation was diagnosed if it was found during at least half of systole and with a minimum velocity of 80 cm/s.

Statistical analysis

In each trisomy 21 and normal pregnancy, the measured NT was converted to a difference from the normal median for CRL (delta value), established from a previous screening study of 96 127 singleton pregnancies[7,8], and the maternal serum free β-hCG and PAPP-A measurements were converted to multiples of the median (MoM) values derived from previous studies of unaffected pregnancies and corrected for maternal weight, ethnic origin, and smoking status[9–13]. Statistical analysis of data was performed with Analyse-It (Smart Software, Leeds, UK), a statistical software add-in for Microsoft Excel.

In order to simulate the performance of population screening using the four markers (fetal NT, maternal serum free β-hCG, PAPP-A and tricuspid regurgitation) in combination with maternal age, we used the maternal age distribution of England and Wales and standard statistical modeling techniques[14,15]. We used the distribution parameters and inter-relationships for the first three markers from a large well described population of trisomy 21 and normal fetuses[5,16] and the frequency of tricuspid regurgitation in the trisomy 21 and normal fetuses from this study. We assumed that the lack of a significant association between maternal serum metabolites and absence of tricuspid regurgitation in the trisomic fetuses we identified by the combined approach would apply equally to those trisomic fetuses that were in the screen negative group. A series of 15 000 delta NT values, and MoMs for free β-hCG and PAPP-A for the unaffected pregnancy group and for the trisomy 21 group were selected at random from within the Gaussian distribution of each marker in each pregnancy group. To take into account the recently described relationship between CRL, NT and incidence of tricuspid regurgitation[3], we randomly assigned to the 15 000 data sets a CRL value with 25% of cases in the range 44–54 mm, 50% in the range 55–69 mm and 25% in the range 70–84 mm, this being the distribution seen in our center over the past 5 years. From the CRL and NT we calculated a delta NT and then randomly assigned the presence or absence of tricuspid regurgitation based on the CRL and delta NT using the frequencies described by Faiola *et al.*[3]. From these data sets we firstly calculated a likelihood ratio for trisomy 21 based on the described relationship between tricuspid regurgitation, NT and CRL[3]. We then calculated a likelihood ratio for trisomy 21 based on the described relationship of delta NT and then a likelihood ratio for trisomy 21 based on the Gaussian distributions of the two biochemical markers[5,16]. The tricuspid regurgitation likelihood ratio was then multiplied by the delta NT and biochemistry likelihood ratios to provide an integrated likelihood ratio for all four markers. The integrated

likelihood ratios for the two pregnancy populations were then used together with the age-related risk of trisomy 21 at 12 weeks' gestation to calculate the expected detection rate of affected pregnancies at various false positive rates in a population with the maternal age distribution of pregnancies in England and Wales[6,14].

In order to model the performance of the two-stage approach to screening[1] using initially NT and maternal serum biochemistry followed by tricuspid regurgitation in those with an intermediate risk, we followed the procedure outlined by Wright *et al.*[17].

RESULTS

The demographic characteristics of the trisomy 21 and normal groups are summarized in Table 1. The median delta NT, and free β-hCG and PAPP-A MoM, adjusted for maternal weight, ethnic group and cigarette smoking in the trisomy 21 and normal fetuses, according to the presence or absence of tricuspid regurgitation, are shown in Table 2 and illustrated in Figure 1. Comparison of the $\log_{10}$ MoMs by *t*-test of unequal variance demonstrated no significant difference between those with and those without tricuspid regurgitation.

The modeled detection rates of trisomy 21 for fixed false positive rates of 1, 2 and 5% in screening by maternal age, fetal NT thickness and maternal serum free β-hCG and PAPP-A and by maternal age, fetal NT, serum biochemistry and tricuspid regurgitation are shown in Table 3.

Table 1 Demographic characteristics of trisomy 21 and normal pregnancies

	Trisomy 21 (n = *77*)	*Normal* (n = *232*)
Maternal age, median (range), years	37 (24–45)	37 (25–46)
Maternal weight, median (range), kg	63 (44–105)	63 (40–104)
Ethnic origin, *n* (%)		
Caucasian	70 (90.9)	225 (97.0)
Afro-Caribbean	2 (2.6)	3 (1.3)
Asian	4 (5.2)	3 (1.3)
Oriental	1 (1.3)	1 (0.4)
Cigarette smoker, *n* (%)	7 (9.1)	9 (3.9)
Nulliparity, *n* (%)	29 (37.7)	62 (26.7)
Assisted reproduction, *n* (%)	4 (5.2)	17 (7.3)
Fetal CRL, median (range), mm	64 (50–79)	64 (45–84)
Tricuspid regurgitation, *n* (%)	57 (74.0)	16 (6.9)

CRL, crown–rump length.

In the two-stage approach the risk after the first-stage screening was 1 in 100 or more in 2.4% of the pregnancies with normal fetuses and in 81.5% of those with trisomy 21 (Table 4). In the 16.1% of women with an intermediate risk (which included 16% of the pregnancies with trisomy 21) who had second-stage screening with tricuspid regurgitation, the modified risk became 1 in 100 or more in 1.3% (or 0.21% of the total) of the pregnancies

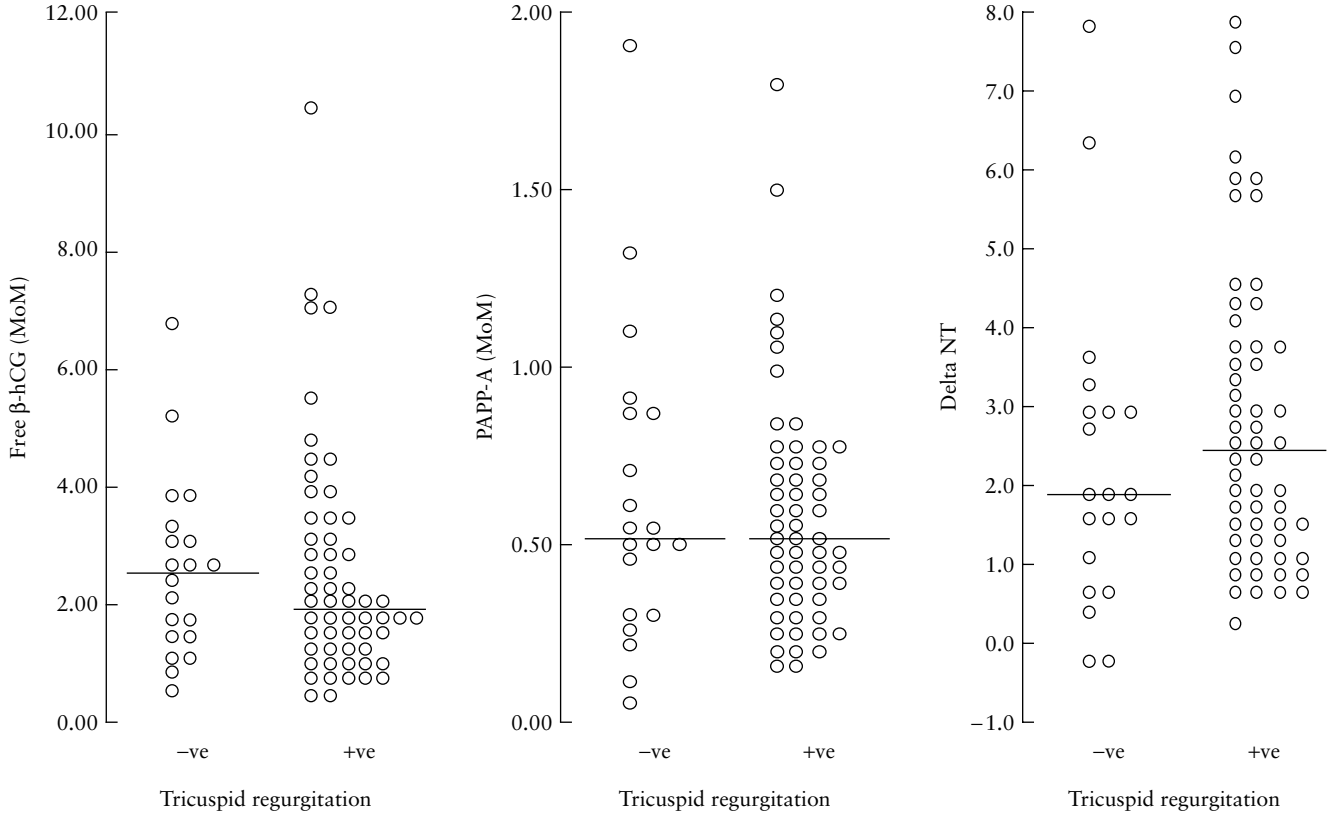

Figure 1 Maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) and delta nuchal translucency (NT) thickness in pregnancies with fetal trisomy 21 in the absence and presence of tricuspid regurgitation. MoM, multiples of the median. Horizontal lines represent the medians.

Table 2 Median (95% CI) of delta nuchal translucency thickness and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in trisomy 21 and chromosomally normal pregnancies according to the presence or absence of tricuspid regurgitation

	Tricuspid regurgitation		*Probability of difference*
	Present	*Absent*	
Trisomy 21			
Delta nuchal translucency (mm)	2.19 (1.57–2.91)	1.76 (0.92–2.78)	0.155
Free β-hCG (MoM)	1.91 (1.64–2.36)	2.42 (1.44–3.11)	0.484
PAPP-A (MoM)	0.57 (0.44–0.69)	0.56 (0.30–0.88)	0.337
Normal			
Delta nuchal translucency (mm)	0.21 (−0.03 to 0.91)	0.12 (−0.07 to 0.21)	0.299
Free β-hCG (MoM)	0.88 (0.50–2.75)	1.03 (0.87–1.13)	0.297
PAPP-A (MoM)	1.05 (0.58–1.52)	1.00 (0.85–1.12)	0.322

MoM, multiples of the median.

Table 3 Modeled detection rates of trisomy 21 for fixed false positive rates of 1, 2 and 5% in screening by fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), and by fetal NT, serum biochemistry and tricuspid regurgitation

	Detection rate (%)	
False positive rate (%)	*NT, free β-hCG and PAPP-A*	*NT, free β-hCG, PAPP-A and tricuspid regurgitation*
1	75	87
2	80	90
5	90	95

Table 4 Detection rate and false positive rate with a two-stage approach to screening for trisomy 21. In the first stage, screening is by fetal nuchal translucency thickness and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) and invasive testing is carried out if the risk is 1 in 100 or more. Those with an intermediate risk (1 in 101 to 1 in 1000) have second-stage assessment with examination of tricuspid flow. If there is tricuspid regurgitation the risk becomes 1 in 100 or more and invasive testing is carried out

Screening	*Detection rate (%)*	*False positive rate (%)*
First stage	81.5	2.4
Second stage	9.6	0.2
Total	91.1	2.6

with normal fetuses and in 60% (or 9.6% of the total) of those with trisomy 21. Therefore, the overall detection rate would be 91.1% and the false positive rate would be 2.6%.

DISCUSSION

The findings of this study confirm that firstly, in about 70% of fetuses with trisomy 21 there is tricuspid regurgitation at 11 to 13 + 6 weeks' gestation and secondly, trisomy 21 is associated with increased fetal NT, elevated levels of maternal serum free β-hCG and reduced levels of maternal serum PAPP-A. Furthermore, the data demonstrate that in both the trisomy 21 and the chromosomally normal pregnancies the levels of maternal serum free β-hCG and PAPP-A are independent of the presence or absence of tricuspid regurgitation. Assessment of tricuspid regurgitation can effectively be combined with fetal NT thickness and maternal serum biochemistry to improve the detection rate from about 90 to 95%, for the same false positive rate of 5%. Alternatively, the detection rate of 90% could be achieved with a 60% reduction in the false positive rate, from 5 to 2%.

The diagnosis of tricuspid regurgitation at 11 to 13 + 6 weeks' gestation is based on the use of pulsed-wave Doppler rather than color-flow mapping, and the presence of regurgitation during at least half of systole and with a minimum velocity of 80 cm/s[3]. These criteria are essential to avoid the erroneous diagnosis of tricuspid regurgitation in the presence of a jet of up to 50 cm/s produced by overlying aortic or pulmonary arterial blood flow, or by the short reverse spike generated by the closure of the valve cusp itself. An experienced fetal echocardiographer can obtain an apical four-chamber view, with the spine either anterior or posterior, for adequate assessment of the flow profile within a 5-min period in more than 95% of cases. An additional benefit of incorporating tricuspid regurgitation in routine screening for trisomy 21 is the potential for increased detection of cardiac defects. This is likely to be the inevitable consequence of obtaining a good four-chamber view of the fetal heart, as well as the finding that tricuspid regurgitation substantially increases the risk for cardiac defects[3].

Assessment for tricuspid regurgitation is time-consuming and requires highly skilled operators and it is therefore unlikely that, at present, this assessment will be incorporated into the routine first-trimester scan. An alternative approach to routine assessment of tricuspid regurgitation in all cases is to reserve this examination for the subgroup of pregnancies with an intermediate risk after combined fetal NT and maternal serum free β-hCG and PAPP-A screening, which constitutes only one sixth of the total population[1]. In a previous screening study by

fetal NT and maternal serum free β-hCG and PAPP-A in 75 821 singleton pregnancies, the risk was less than 1 in 1000 in 82% of the chromosomally normal fetuses and in 3% with trisomy 21 (low-risk group), between 1 in 101 and 1 in 1000 in 16% of the normal fetuses and in 15% with trisomy 21 (intermediate-risk group) and 1 in 100 or more in 2% of the normal fetuses and in 82% with trisomy 21 (high-risk group)[1]. In the two-stage screening approach, the 16% of the patients with an intermediate risk have further assessment by Doppler ultrasonography across the tricuspid valve. As demonstrated in the present study, with two-stage screening, for a false positive rate of 2.6% the detection rate of trisomy 21 would be 91.1%.

In both the integrated and the two-stage approaches to first-trimester assessment of risk the performance of screening by including examination of flow across the tricuspid valve is similar to that of including examination for the presence or absence of the nasal bone[1,18–20]. The extent to which further improvement can be achieved by examining both the tricuspid flow and nasal bone remains to be determined. In the meantime, only one of the two markers can be examined and the choice would depend on fetal position and the skill of the individual sonographer.

CONCLUSIONS

Doppler examination of tricuspid flow can improve the performance of first-trimester screening for trisomy 21 provided by fetal NT thickness and maternal serum biochemistry. Examination of the fetal heart can be undertaken in all cases, with the added advantage of early diagnosis of major cardiac defects. Alternatively, if the expertise in fetal echocardiography is limited, a two-stage approach can be adopted, whereby assessment of tricuspid regurgitation is confined to about one sixth of the population that constitute the intermediate-risk group after screening by fetal NT and serum free β-hCG and PAPP-A.

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