

Second-trimester levels of pregnancy-associated plasma protein-A and free β -hCG in pregnancies with trisomy 13

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Objective To examine the levels of free β -human chorionic gonadotrophin (free β -hCG) and pregnancy-associated plasma protein-A (PAPP-A) in second-trimester maternal serum from pregnancies affected by trisomy 13 and compare these with the known reduced levels of these markers in first-trimester cases in an attempt to better understand the pathophysiology of changes in marker levels in chromosomally abnormal pregnancies between the first and second trimester.

Methods Using the Kryptor immunoassay system, we measured free β -hCG and PAPP-A in 32 singleton pregnancies affected by trisomy 13 between 14 and 20 weeks of gestation. Using medians established in a previous study, these results were compared against 450 normal singleton pregnancies over the same gestational range. The data were combined with data from 82 cases of trisomy 13 previously examined in the first trimester (11–13 weeks) and an analysis of analyte trend was performed.

Results The median free β -hCG in multiples of the appropriate gestational median (MoM) in the second-trimester samples was not significantly different from the controls (1.15 (95% CI 0.827–1.651) vs 1.00). The median PAPP-A MoM in the second-trimester samples was significantly lower ($p < 0.001$) than in controls (0.25 (95% CI 0.164–0.373) vs 1.00). Seventy-eight percent of cases were below the 5th centile of normal for PAPP-A. The combined cases in the first trimester had a median free β -hCG MoM of 0.58 (95% CI 0.454–0.668) and a median PAPP-A MoM of 0.26 (95% CI 0.218–0.320). For PAPP-A, there was no significant change in median across the gestational period of 11 to 20 weeks, whilst for free β -hCG, there was a significant increase with gestation ($r = 0.458$, $p < 0.001$).

Conclusions Although PAPP-A levels are reduced in trisomy 13 pregnancies in the second trimester, this isolated lower marker value is unlikely to be of value in screening for trisomy 13 in the second trimester. The aetiology of reduced levels of PAPP-A in cases with trisomy 13 may be similar to that in cases with trisomy 18, but different from that in cases with trisomy 21 since the temporal pattern in trisomies 13 and 18 are different from that in trisomy 21. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: trisomy 18; trisomy 21; prenatal screening; first trimester; biochemical screening

INTRODUCTION

In the first trimester of pregnancy, a low level of maternal serum pregnancy-associated plasma protein-A (PAPP-A) and a raised maternal serum free β -hCG has been shown to be associated with the presence of a fetus affected by trisomy 21 (Spencer *et al.*, 1999a). In the second trimester of pregnancy, while maternal serum free β -hCG levels are still raised in pregnancies affected by trisomy 21 (Spencer, 1991; Spencer *et al.*, 1992; Macri *et al.*, 1994; Spencer, 1999), the low levels of maternal serum PAPP-A are no longer evident (Spencer *et al.*, 1994), and levels approach normal by the 16th to 17th week of gestation (Berry *et al.*, 1997; Spencer *et al.*, 2002).

In cases of trisomy 18, the levels of maternal serum PAPP-A and free β -hCG are both low in the first trimester (Tul *et al.*, 1999) and remain low throughout the second trimester, with PAPP-A levels progressively

falling to even lower values across this period (Spencer *et al.*, 1999b; Bersinger *et al.*, 1999). Indeed, the use of PAPP-A as a two-stage screening program in the second trimester is predicted to detect 82% of cases of trisomy 18 at a 0.1% false-positive rate (Spencer *et al.*, 1999b; Muller *et al.*, 2002).

In trisomy 13, the commonly used maternal serum biochemical markers in the second trimester are largely unsuccessful in identifying cases of trisomy 13 (Saller *et al.*, 1999). However, in the first trimester, maternal serum PAPP-A and free β -hCG levels are reduced and, in conjunction with fetal nuchal translucency (NT), it has been suggested that over 90% of cases could be identified for a very low false-positive rate (Spencer *et al.*, 2000a, b; Spencer and Nicolaides, 2003).

In this study, we have examined the pattern of these first-trimester biochemical markers of trisomy 13 in pregnancies affected by trisomy 13 in the second trimester in an attempt to better understand the pathophysiology of changes in marker levels in chromosomal abnormalities between the first and second trimester and to examine the trends in PAPP-A levels in trisomy 13 pregnancies in the second trimester.

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METHODS

The trisomy 13 population comprised women who presented through the neural tube defect and Down syndrome screening programmes in two centres. Serum collected from each woman was stored at -20°C after routine screening with either AFP and free β -hCG or AFP and total hCG assays. The samples plus analytical and clinical data were collated on the basis of abnormal birth outcome confirmed cytogenetically or cytogenetic confirmation of an abnormality after mid-trimester amniocentesis. Thirty-two singleton pregnancies affected by trisomy 13 were available for the study. Of the eight cases with an AFP MoM greater than 2.5, three cases had anencephaly, three had an open spina bifida and one had a ventral wall defect. Samples had been frozen and thawed a maximum of three times. Gestational dating was in weeks and days by the best estimate of gestation at the time of sampling. Control samples identified from one centre used in a previous study of trisomy 18 (Spencer *et al.*, 1999b) were examined and found to be matched appropriately for maternal age, gestational age and length of storage, and these formed the comparison control group. The study population data are summarised in Table 1.

Maternal serum free β -hCG, PAPP-A and AFP were measured using the Kryptor analyser—a rapid random access immunoassay analyser (Brahms AG, Berlin). The analytical performance of these assays has been previously reported (Spencer *et al.*, 1999a, b). The data from the control population from our previous study was used for comparison of all three analytes (Spencer *et al.*, 1999b).

Data for maternal serum free β -hCG and PAPP-A in pregnancies with trisomy 13 identified in the first trimester were available from a previous study, which included 42 cases (Spencer and Nicolaides, 2003). This data set was supplemented with a series of samples collected as part of prospective screening in two one-stop screening (OSCAR) centres (Bindra *et al.*, 2002; Spencer *et al.*, 2003). In total, 82 first-trimester data sets were available.

RESULTS

All analyte measurements were converted to multiples of the median (MoM) using the previously derived medians for normal pregnancies (Spencer *et al.*, 1999b). The median serum AFP level in trisomy 13 pregnancies was not significantly different from that in unaffected

pregnancies, with a median MoM of 1.09 (95 per cent confidence interval 95% CI 0.86 to 1.58) and a range of 0.59 to 7.48. The median serum-free β -hCG level in the trisomy 13 pregnancies was not significantly different from that in unaffected pregnancies, with a median MoM of 1.15 (95% CI 0.83 to 1.65) and a range of 0.28 to 7.81. The median serum PAPP-A level in the trisomy 13 pregnancies was significantly lower (unpaired *t*-test of unequal variance on log₁₀ MoM, $p < 0.001$) than in unaffected pregnancies, with a median MoM of 0.25 (95% CI 0.16 to 0.37), a range of 0.01 to 1.53 and log₁₀ SD of 0.54. The results of the individual cases are shown in Figures 1, 2 and 3. The 5th centile of the PAPP-A MoM distribution in unaffected pregnancies was 0.42 MoM. Seventy-eight percent of the trisomy 13 cases (25/32) had PAPP-A MoMs below this level.

In the first trimester, levels of PAPP-A and free β -hCG are low in cases of trisomy 13 (Spencer *et al.*, 2000a, b; Spencer and Nicolaides, 2003). To assess the variation of analyte levels through the first and second trimester, we combined the second-trimester data from this present study with those of the first-trimester studies (Spencer *et al.*, 2000a, b; Spencer and Nicolaides, 2003), supplemented by other cases from routine first-trimester screening. The median PAPP-A level in the 82 first-trimester cases was 0.26 MoM (95%

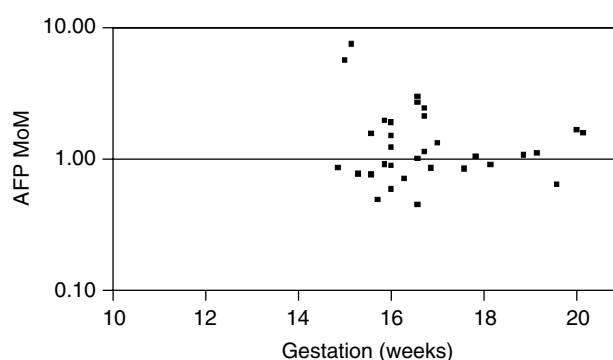


Figure 1—Variation of AFP MoM in cases of trisomy 13 in the second trimester

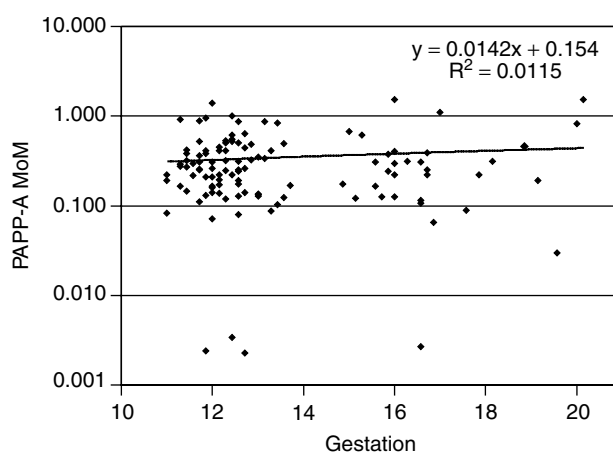


Figure 2—Temporal changes of PAPP-A MoM across the first and second trimesters in 114 cases of trisomy 13

Table 1—Parameters of the second-trimester trisomy 13 and control populations

	T13 population median (range)	Control population median (range)
Number	32	450
Gestational age (weeks)	16 (14–19)	16 (14–19)
Maternal age (years)	32 (21–41)	29 (20–44)
Storage time (years)	4.1 (0.2–6.5)	3.6 (0.8–6.0)

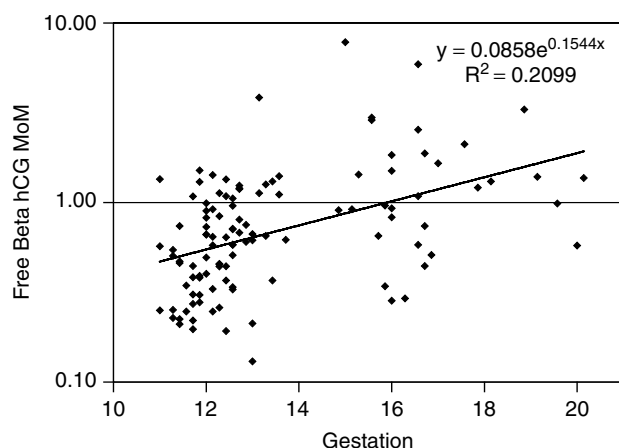


Figure 3—Temporal changes of free β -hCG MoM across the first and second trimesters in 114 cases of trisomy 13

CI 0.22–0.32), whilst that for free β -hCG was 0.58 MoM (95% CI 0.45–0.67). For PAPP-A, there was no significant change in MoM levels in the trisomy 13 cases across the 11- to 20-week period with the median being reduced in both trimesters and no correlation observed with gestational age ($r = 0.107$) as shown in Figure 2. For free β -hCG, a significant increase in MoM levels was observed in the trisomy 13 cases with increasing gestational age ($r = 0.458$) as shown in Figure 3, such that by 20 weeks the median MoM would be expected to be close to 2 MoM.

DISCUSSION

Trisomy 13 is the third most common autosomal trisomy with a birth incidence of 1 in 12 500. At 14 weeks, the incidence is of the order of 1 in 5000, which compares with an incidence of 1 in 500 for trisomy 21. The *in utero* characteristic feature is growth deficiency and this trisomy is associated with multiple congenital anomalies, including central nervous system disorders, neural tube defects, congenital heart defects and cleft palate. The median survival time of infants is less than 1 month, with more than 80% dying in the first month and only 3% surviving 6 months. Despite this lethality, the option of prenatal diagnosis would allow parents to consider a variety of options regarding continuous or supportive care of an affected pregnancy.

In the second trimester of pregnancy, one study has examined levels of AFP, total hCG and unconjugated oestriol (UE3) in trisomy 13 (Saller *et al.*, 1999). In 28 cases (including 3 with NTD), the median AFP level was 1.35 MoM (1.34 excluding these cases) and this was not statistically significantly different from the controls. The total hCG median was 0.90 MoM, which also was not significantly different from the controls. A significant difference, however, was found for UE3, which gave a median MoM of 0.71. In our series, the median AFP MoM of 1.09 was similar to the published series, whilst the free β -hCG MoM of 1.15 was not

significantly different from controls. Unlike in cases with trisomy 21, where the clinical discrimination of PAPP-A deteriorates with advancing gestation, in cases with trisomy 13, the lower median MoMs in the first trimester are also apparent in the second trimester. Unlike trisomy 18, levels do not decline further in the second trimester, but are maintained at the median levels seen in the first trimester. Watanabe *et al.* (2002) reported PAPP-A results in four second-trimester cases with trisomy 13 at 15 and 16 weeks and found median values to be reduced to 0.73 MoM, much higher than those found in our larger series. Thus, with reference to the reported markers, Saller *et al.* (1999) concluded that it was not possible to design a screening protocol for fetal trisomy 13 as part of second-trimester maternal serum screening programme, although this conclusion was based on very little Inhibin A data. In the four cases reported by Watanabe *et al.* (2002), the median Inhibin A level was 1.14 MoM. Hsu *et al.* (2003) reported one case with a MoM of 0.89. Cuckle *et al.* (1999) reported six cases with a median Inhibin A MoM of 2.21 and 0.734 for UE3 and 1.08 for free β -hCG. Wenstrom *et al.* (1998) reported that a combination of AFP and Dimeric Inhibin A detected autosomal trisomies other than Down syndrome at a rate superior to that of other combinations. Although this study included seven cases with trisomy 13 and claimed a detection rate of 71% (5/7 cases), no data on individual marker levels were provided. However, one would suspect that given other observations of a normal AFP in such cases, the Dimeric Inhibin A levels were most certainly elevated, as suggested by the data of Cuckle *et al.* (1999).

Unlike trisomy 18 in the second trimester, it seems that there is no combination of commonly used serum markers that can be used to preselect pregnancies for secondary analysis with PAPP-A to enable identification of those with trisomy 13. With only consistently reduced levels of UE3 and normal to raised levels of Dimeric Inhibin A reported in cases with trisomy 13, much more marker data would be needed to develop a screening protocol to select pregnancies of interest for further evaluation.

It is interesting, however, that of the types of aneuploid pregnancies with low PAPP-A levels in the first trimester (trisomy 21, trisomy 18 and trisomy 13), only the levels in trisomy 21 become normal by the 16 to 17th week in the second trimester. This suggests that the aetiology of reduced PAPP-A is different in cases with trisomy 13 and 18 compared to trisomy 21. Whether the persistently reduced PAPP-A level found across the first and second trimesters in trisomy 18 and trisomy 13 pregnancies is simply acting as a marker of the relative non-viability of these conditions compared to trisomy 21 or is a result of the modified placental synthesis and release of PAPP-A directed by specific chromosomes will require further study.

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