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Maternal serum levels of dimeric inhibin A in pregnancies affected by trisomy 21 in the first trimester

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Dimeric inhibin A was measured in maternal serum samples from 45 pregnancies affected by trisomy 21 and 493 samples from unaffected pregnancies at 10–14 weeks of gestation. Inhibin A levels in affected pregnancies were compared with levels of free β-hCG and PAPP-A in the same series. In the trisomy 21 group, the median multiple of the median (MoM) inhibin A was not significantly elevated (1.28 vs 1.00) with only 15.5% being above the 95th centile. In contrast, the median MoM free β-hCG was significantly increased (2.05 vs 1.00) with 36% above the 95th centile and PAPP-A was significantly reduced (0.49 vs 1.00) with 42% below the 5th centile. Inhibin A levels in the trisomy 21 group were significantly correlated with gestational age such that median levels rose from 1.04 at 11 weeks to 1.30 at 12 weeks and 1.67 at 13 weeks. These findings suggest that first trimester biochemical screening for trisomy 21, which is currently optimised using maternal serum free β-hCG and PAPP-A and fetal nuchal translucency, will not benefit from the inclusion of inhibin A. Copyright © 2001 John Wiley & Sons, Ltd.

KEY WORDS: inhibin; trisomy 21; first trimester; aneuploidy; free β-hCG; PAPP-A; prenatal screening

INTRODUCTION

In trisomy 21 pregnancies maternal serum free β-hCG is increased both in the first and second trimesters of pregnancy (Macri *et al*., 1990; Spencer, 1991; Spencer *et al*., 1992). Free β-hCG has been combined with α-fetoprotein (AFP) in the second trimester (Spencer, 1999) and pregnancy associated plasma protein-A (PAPP-A) and fetal nuchal translucency in the first trimester (Spencer *et al*., 1999) to provide successful screening programs for trisomy 21 and other chromosomal anomalies.

The increase in maternal serum hCG in trisomy 21 pregnancies may be mediated by increased placental production (Eldar-Geva *et al*., 1995; Goshen *et al*., 1999; Knofler, 1999). Control of hCG production in the placenta may well involve inhibin, which is known to inhibit placental hCG synthesis *in vitro* through modulated placental Gonadotrophin releasing hormone (GnRH) release (Petraglia *et al*., 1989). Early studies of total immunoreactive inhibin in pregnancies affected by trisomy 21 in the second trimester reported that although levels were increased there was a wide standard deviation and a high degree of correlation with hCG (van Lith *et al*., 1992; Spencer *et al*., 1993; Cuckle *et al*., 1994). It was therefore suggested that addition of inhibin did not improve serum screening for trisomy 21. However, improvements in assay specificity (Groome and O'Brien, 1993) led to the conclusion that addition of inhibin A in second trimester serum screening improves the rate of detection of trisomy 21 on average by 4–7% (Aitken *et al*., 1996; Cuckle *et al*., 1996; Lambert-Messerlian *et al*., 1996; Spencer *et al*., 1996; Wald *et al*., 1996a; Wallace *et al*., 1996).

Biochemical screening is now moving into the first trimester in conjunction with the use of fetal nuchal translucency thickness. In the present study we investigate the possible value of maternal serum inhibin A in screening for trisomy 21 at 10–14 weeks of gestation.

PATIENTS AND METHODS

At the Harris Birthright Research Centre and at Harold Wood Hospital maternal serum samples were collected at 10–14 weeks of gestation as part of screening for trisomy 21 and other chromosomal anomalies by maternal age, fetal nuchal translucency thickness and maternal serum free β-hCG and PAPP-A (Spencer *et al*., 1999, 2000a). All serum was aliquoted and stored at -20°C within 1 h of collection. Institutional research ethics approval had been granted for further research on stored excess clinical material.

From the stored samples, 45 cases of trisomy 21 and 493 control pregnancies were selected, matched for gestational age and length of storage. Maternal serum inhibin A was measured in all samples in duplicate using a quantitative enzyme immunoassay technique (Serotec, Kidlington, UK). The inter-assay precision was 8% and the intra-assay precision was 9% at 70 pg/ml, 4% and 4.5% at 200 pg/ml, and 4% and 5% at 1100 pg/ml. Maternal serum PAPP-A and free β-hCG were measured using the CIS Kryptor system as described previously (Spencer *et al*., 1999).

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Statistical analysis

In the unaffected pregnancies regression analysis was used to establish median inhibin A concentrations for each gestation in days. All measurements, both in the trisomy 21 and unaffected pregnancies, were then expressed as multiples of the median (MoM). Similarly, MoM values were established for free β-hCG and PAPP-A with correction for maternal weight (Spencer *et al.*, 1999). Analyse-It software (Smart Software, Leeds, UK) was used for statistical analysis and normality of the distributions was examined by the Kolmogorov-Smirnov test. Significance of marker levels in the trisomy 21 group was analysed by Student's t test of unequal variance on Gaussian confirmed distributions.

RESULTS

In the unaffected pregnancies maternal serum inhibin A decreased exponentially with gestation (Table 1). The characteristics of the trisomy 21 and unaffected pregnancies are summarised in Table 2. The median values in trisomy 21 pregnancies were 1.277 MoM for inhibin A, 2.046 MoM for free β-hCG and 0.487 MoM for PAPP-A. In trisomy 21 16% (7/45) had inhibin A levels above the 95th centile, 36% (16/45) had free β-hCG above the 95th centile and 42% (19/45) had PAPP-A levels below the 5th centile of normal.

Log_{10} transformation was necessary to normalise the data for inhibin-A, free β-hCG and PAPP-A in both the trisomy 21 and unaffected pregnancies. The mean $\log_{10}$ inhibin MoM in the trisomy 21 group was 0.101 with a $\log_{10}$ SD of 0.3485 compared with 0.001 and 0.1936, respectively, in the unaffected group. Comparing the trisomy 21 $\log_{10}$ MoMs with those of the unaffected group the difference was not significant ($p = 0.0547$). When the individual marker MoMs were compared with each other a significant correlation was found between inhibin A and free β-hCG ($r = 0.3852$) and between inhibin A and PAPP-A ($r = 0.4241$) in the trisomy 21 group. In the control group a small but significant correlation was observed between inhibin A and free β-hCG ($r = 0.242$) but not between inhibin A and PAPP-A ($r = 0.076$).

In trisomy 21 pregnancies the MoM values of inhibin A increased significantly with gestation ($r = 0.332$). When the individual results were analysed by completed weeks of gestation, the median MoM rose from 1.036 [95% confidence interval (CI) = 0.675–1.672] at 11 weeks ($n = 11$) to 1.299 (95% CI = 1.056–1.472) at 12 weeks ($n = 26$) and 1.667 (95% CI = 1.156–1.838) at 13 weeks ($n = 8$). The individual results are shown in Figure 1.

Table 1—Observed and regressed medians for inhibin A in the first trimester

Gestation (days)	Observed pg/ml (number of controls)	Regressed pg/ml
70		268
73.5	217 (40)	252
77		236
80.5	232 (152)	221
84		207
87.5	189 (217)	194
91		182
94.5	194 (84)	171

Table 2—Characteristics of the trisomy 21 and unaffected pregnancies

	Trisomy 21 ($n = 45$)	Unaffected ($n = 493$)
Maternal age (years)	36.62 (SD 5.28)	28.1 (SD 5.2)
Maternal weight (kg)	67.5 (SD 10.6)	68.0 (SD 13.6)
Caucasian (%)	41 (91%)	430 (87.2%)
Cigarette smokers (%)	5 (11.1%)	78 (15.8%)
Primigravidae (%)	13 (28.9%)	240 (48.7%)
Gestational age (weeks)	12 (SD 0.6)	12 (SD 0.8)

DISCUSSION

In the present study of 45 trisomy 21 pregnancies at 10–14 weeks of gestation the median MoMs for maternal serum inhibin A, free β-hCG and PAPP-A were 1.28, 2.05 and 0.49. Furthermore, there was a significant association between free β-hCG and inhibin A levels. Consequently, inhibin A does not improve the 90% sensitivity (at a 5% false-positive rate) of screening by a combination of maternal serum free β-hCG and PAPP-A and fetal nuchal translucency at 10–14 weeks (Spencer *et al.*, 1999).

The finding that in trisomy 21 pregnancies there is a small increase in maternal serum inhibin A concentration is compatible with previous reports in the first trimester. Thus, in a total of 235 first trimester trisomy 21 pregnancies the median MoM for inhibin A is 1.51 (Table 3), compared to 1.85 in the second trimester (Table 4). Furthermore, the standard deviation of inhibin A in cases of trisomy 21 in the first trimester is wider than that found in the second trimester (0.3485 vs 0.2988) (Spencer *et al.*, 1996).

The difference in maternal serum inhibin A between trisomy 21 and unaffected pregnancies increases with gestation. This is also true for total hCG (Spencer *et al.*, 2000b). In contrast, the difference in PAPP-A decreases with gestation. The underlying mechanisms for these temporal changes and the regulation of the placental production of these peptides are not established. However, there is evidence from studies of cultured trophoblasts that inhibin suppresses the release of hCG but this suppression is gestation dependent with no effect observed in the first trimester (Mersol-Barg *et al.*, 1990).

The small increase in maternal serum inhibin A in trisomy 21 pregnancies at 10–14 weeks of gestation is unlikely to be exploited in improving the sensitivity of screening by a combination of free β-hCG, PAPP-A and fetal nuchal translucency.

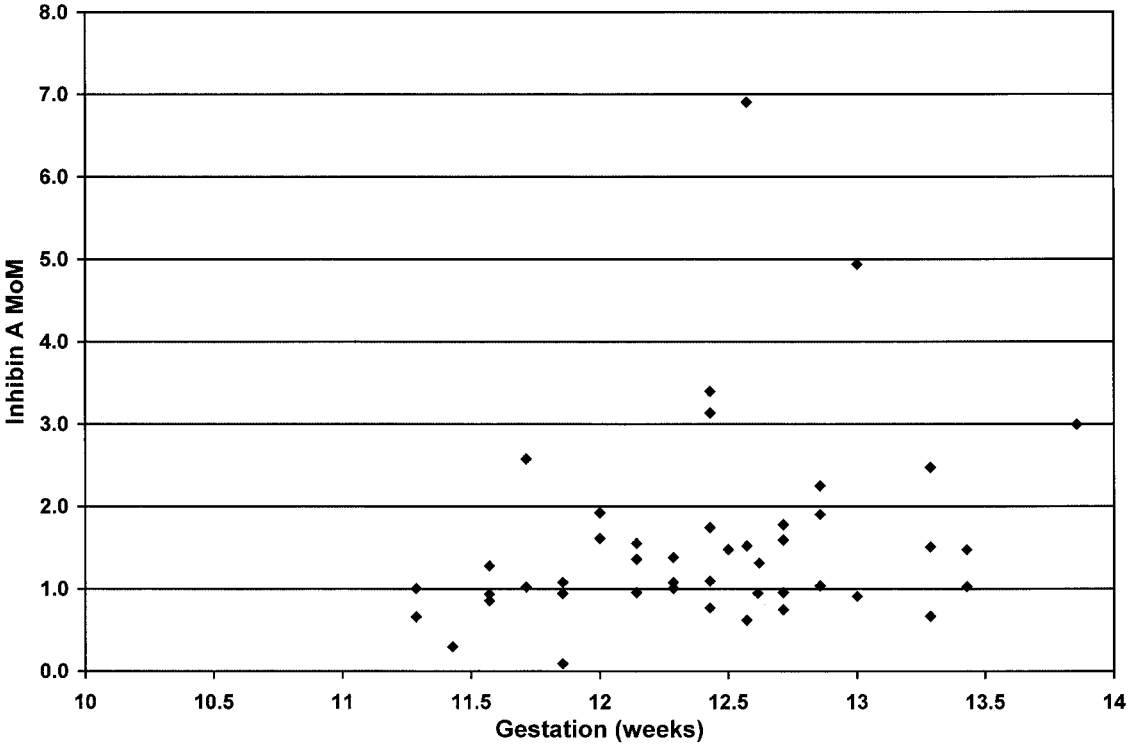

Figure 1—Inhibin A MoM in 45 cases of trisomy 21 across the first trimester

Table 3—Summary of published studies of inhibin A in cases of trisomy 21 in the first trimester

Study	Cases/controls	Median MoM
Wallace *et al.* (1995)	23/89	2.46
Aitken *et al.* (1996)	14/206	1.38
Wald *et al.* (1996b)	77/383	1.19
Noble *et al.* (1997)	76/800	1.51
Resent study	45/493	1.28
Totals	235/1971	1.41

Table 4—Summary of published studies of inhibin A in cases of trisomy 21 in the second trimester

Study	Cases/controls	Median MoM
Wallace *et al.* (1996)	21/150	2.60
Cuckle *et al.* (1996)	56/280	1.62
Aitken *et al.* (1996)	44/202	2.24
Spencer *et al.* (1996)	157/367	1.77
Lambert-Messerlian *et al.* (1996)	20/100	1.95
Wald *et al.* (1996a)	77/385	1.79
Wenstrom *et al.* (1997)	33/313	2.33
Haddow *et al.* (1998)	52/256	2.10
D'antona *et al.* (1998)	43/300	1.53
Lam and Tang (1999)	49/341	1.62
Totals	552/2694	1.85

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