

Serum müllerian-inhibiting substance in Down's syndrome pregnancies

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BACKGROUND: To examine whether maternal serum levels of müllerian-inhibiting substance (MIS) differ in Down's syndrome and unaffected pregnancies. **METHODS:** Case-control study was conducted using stored serum from an antenatal screening programme. Sera from 25 Down's syndrome pregnancies were retrieved from -20°C storage together with 125 unaffected controls individually matched for maternal age, weeks of gestation and duration of storage. Results were expressed in multiples of the gestation-specific median value (MoM) in controls. **RESULTS:** The median value in Down's syndrome pregnancies was 0.83 MoM ($P = 0.77$, two-tail Wilcoxon rank sum test). Among unaffected pregnancies, there was a statistically significant correlation between MIS and pregnancy-associated plasma protein-A ($P < 0.05$). MIS levels were elevated in pregnancies where assisted reproduction techniques had been used. **CONCLUSION:** There is no evidence for a substantial reduction in maternal serum MIS levels in Down's syndrome pregnancies. This study provides useful information regarding serum MIS levels in pregnancy.

Key words: antenatal screening/Down's syndrome/marker/MIS/ovarian reserve

Introduction

The cause of the non-disjunction leading to fetal Down's syndrome is not known, but there is evidence that women with affected pregnancies have diminished ovarian reserve. Two studies (Nasser *et al.*, 1999; van Montfrans *et al.*, 1999) have reported elevated serum follicle-stimulating hormone (FSH) levels, on average, whereas a third study found normal results as well as normal inhibin B levels and antral follicle number (Kline *et al.*, 2004). Women who have had a child with Down's syndrome have an earlier menopause (Freeman *et al.*, 2000; Kline *et al.*, 2000; Bartmann *et al.*, 2005), and surgical removal or congenital absence of one ovary is associated with an increased Down's syndrome incidence (Phillips *et al.*, 1995).

Studies suggest that müllerian-inhibiting substance (MIS), also known as anti-müllerian hormone, is a useful serum marker of ovarian reserve and ovarian ageing (Seifer *et al.*, 2002; de Vet *et al.*, 2002; van Rooij *et al.*, 2002, 2005; Fanchin *et al.*, 2003; Hazout *et al.*, 2004; Ficicioglu *et al.*, 2006). Several of these studies support the concept that MIS levels better reflect reproductive decline than traditional serum markers such as FSH, estradiol and inhibin B (van Rooij *et al.*, 2002, 2005; Fanchin *et al.*, 2003; Hazout *et al.*, 2004). Furthermore, it has been shown that serum MIS values have a minimal variation during pregnancy (La Marca *et al.*, 2005a).

MIS is a follicular fluid component produced by granulosa cells of pre-antral and small antral follicles and is believed to reflect the primordial pool of non-growing FSH-independent follicles. As such, it appears to regulate the development of normal follicles and regulates steroidogenesis in granulosa cells (Kim *et al.*, 1992; Visser *et al.*, 2006). In addition, studies have implicated follicular MIS as an inhibitor of granulosa cell proliferation (Kim *et al.*, 1992; Seifer *et al.*, 1993) and, in rodents, oocyte meiosis (Takahashi *et al.*, 1986). Concomitant to the age-related decrease in primordial follicles is a decline in serum MIS. As an increase in maternal age is accompanied by a decrease in primordial follicles and an increased incidence in the birth of children with Down's syndrome, we speculated that there could be an association between maternal serum MIS concentrations and children born with this disorder.

This prompted us to measure MIS, in stored maternal serum samples from Down's syndrome and control pregnancies, collected as part of a nationwide antenatal Down screening programme. We tested the hypothesis that women who had a pregnancy complicated by Down's syndrome would have lower serum MIS levels during that pregnancy than women of similar chronological age who had an unaffected pregnancy. If such an association was noted, MIS could serve as an additional antenatal serum screening marker.

Materials and methods

Serum samples were obtained from a bank of material, which had been collected from women having routine screening by Leeds Antenatal Screening Service (Leeds, UK), using either a test combining first trimester maternal serum pregnancy-associated plasma protein (PAPP)-A, free β -human chorionic gonadotrophin (hCG), α -fetoprotein (AFP) and unconjugated estriol (uE₃) with ultrasound nuchal translucency or a second trimester serum-free β -hCG, AFP and uE₃ test.

Sera from 25 women with a concurrent Down's syndrome pregnancy and 125 unaffected pregnancies were retrieved from -20°C storage and measured for MIS without knowledge of which were affected. The controls were individually matched for maternal age (within 2 years), gestational age (same completed week) and duration of storage (within a month). For each case, there were five controls exactly fulfilling the matching criteria. The gestational ages of the cases were 10 weeks (2), 11 weeks (13), 12 weeks (4), 13 weeks (3) and 14–17 weeks (3). The duration of storage was 1 year (90), 2 years (25) and 3 years (35). One of the controls was a twin pregnancy.

The enzyme-linked immunosorbent assay (ELISA) used to measure human MIS has been described elsewhere (Hudson *et al.*, 1990; Lee *et al.*, 1996). Samples were analysed in duplicate at six serial dilutions; the results reported were the mean of three dilutions falling within the linear portion of the standard curve, constructed using four-parameter logistical curve-fitting DeltaSoft II (BioMetallics, Inc., Princeton, NJ, USA). The sensitivity of the assay was 0.5 ng ml^{-1} ; the intra- and inter-assay coefficients of variation were 9 and 15%, respectively. The MIS ELISA did not recognize LH, FSH, activin, inhibin or TGF- β and did not cross-react with bovine or rodent MIS.

Following usual practice with Down's syndrome screening markers, gestational differences were allowed for by expressing values as multiples of the observed gestation-specific normal median value (MoM). Statistical comparisons between cases and controls were made using the non-parametric Wilcoxon rank sum test.

Results

Figure 1A shows the individual MIS concentration in unaffected singleton pregnancies according to gestation. The overall median was 1.75 ng ml^{-1} and the gestation-specific median values were 1.00, 1.70, 1.80, 3.60 and $1.50\text{ (ng ml}^{-1}\text{)}$ at 10, 11, 12, 13 and 14–17 weeks, respectively (Figure 1B). No simple regression curve fitted this data; therefore, so the observed median for the gestational week or period, rather than a regressed value, was used to calculate MoMs.

Among unaffected singleton pregnancies, 32 (26%) had levels below the sensitivity of the assay. The upper 75th, 90th and 95th centiles were 1.92, 3.12 and 4.00 MoM respectively. Among the Down's syndrome pregnancies, seven (28%) had levels $<0.5\text{ ng ml}^{-1}$, the median value was 0.83 MoM, with upper 75th centile 2.29 MoM and 90th centile 3.88 MoM. There were no statistically significant differences in the distribution of MIS levels between the Down's syndrome and unaffected pregnancies ($P = 0.67$, two-tail Wilcoxon rank sum test).

There was no obvious association between MIS levels and maternal weight. Dividing the unaffected singleton pregnancies into five weight groups (<55 , 55–59, 60–64, 65–69 and $\geq 70\text{ kg}$), the median MIS levels were 0.80 (13 women), 1.18 (24), 0.91 (26), 0.59 (13) and 1.03 (38) MoM, respectively.

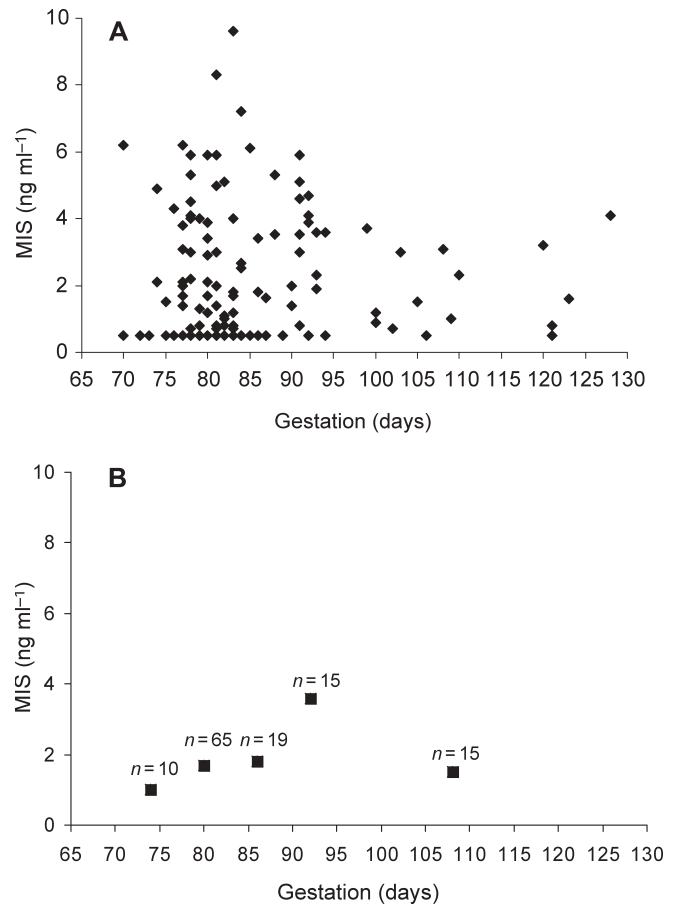


Figure 1. (A) Müllerian-inhibiting substance concentration in unaffected singleton pregnancies according to gestation in days (values below the sensitivity of the assay set to 0.5 ng ml^{-1}) and (B) median concentration in five gestational weeks or periods according to median gestation.

Among unaffected singleton pregnancies, there was a statistically significant correlation between MIS and PAPP-A, after logarithmic transformation of the MoM values and setting the low MIS concentrations to 0.5 ng ml^{-1} ($R = 0.22$, $P < 0.05$). In contrast, MIS and the other Down's syndrome screening markers were uncorrelated.

There was an apparent association between MIS levels and maternal age, even though the maternal age range was narrow. Dividing the unaffected singleton pregnancies into four groups (30–33, 34–37, 38–41 and 42–45 years), the median MIS levels were 2.07 (13 women), 1.00 (37), 0.94 (56) and 0.47 (18) MoM, respectively. The correlation coefficient between log MIS and maternal age was -0.16 ($P = 0.07$).

The MIS level was extremely elevated in the unaffected twin pregnancy (10 MoM), whereas the other Down's syndrome screening markers were close to the expected approximate doubling: PAPP-A, 2.49 MoM; free β -hCG, 2.87 MoM; AFP, 2.74 MoM and uE₃, 2.19 MoM. In this pregnancy, assisted reproduction techniques had been used and it may be relevant that the MIS levels were also relatively increased in the five singleton assisted reproduction pregnancies: 1.24 and 3.88 MoM with Down syndrome; 1.24, 2.73 and 6.53 MoM unaffected.

Discussion

We did not find evidence for a reduction in maternal serum MIS levels in women with concurrent Down's syndrome pregnancies. The median MIS level in Down's syndrome pregnancies was 0.83 MoM, compared with 1.00 MoM in unaffected pregnancies, but there was no statistically significant difference in the distribution of results between cases and controls. We examined only a relatively small series as there are practical difficulties in obtaining samples from a large number of Down's syndrome pregnancies. We cannot exclude the possibility that further larger studies might demonstrate a reduction.

Moreover, the statistical power of our study would have been reduced by the large number of results which fell below the sensitivity of the assay. Impaired statistical power would also have contributed to the observed trend in declining MIS levels with increasing maternal age, which does not reach statistical significance.

Although our principal aim is to examine levels in pregnancies affected by Down's syndrome, these data also provide useful general information on the influence of pregnancy on MIS production.

Two studies have compared MIS concentrations in maternal serum with non-pregnant levels (Al-Qahtani *et al.*, 2005; La Marca *et al.*, 2005a). One of them observed a median in 27 first trimester pregnancies only 5% higher than in 15 non-pregnant women during the follicular phase of the menstrual cycle; in 13 second trimester pregnancies, it was 26% higher, whereas in 13 third trimester pregnancies, the increase was 8% (La Marca *et al.*, 2005a). The second study found the average level in 28 first trimester cases to be two to three times higher than in 12 non-pregnant women (Al-Qahtani *et al.*, 2005). Although we did not have a control group of non-pregnant women, we did observe a general tendency for MIS levels to increase with gestation. However, despite the current study having the largest number of MIS results on pregnant women, the tendency to increase did not reach statistical significance ($P = 0.30$ for weighted quadratic regression of normal median on gestation).

If there was fetoplacental production of MIS, a negative association between maternal serum levels and maternal weight might have been expected. Unless there is a feedback mechanism, the same fetoplacental mass would yield a lower concentration in a large woman, with a larger blood and fluid volume, than in a smaller woman. This effect is clearly demonstrable with all the Down's syndrome markers and is lowest for uE₃ where there are sources of production in maternal fat, independent of the fetus. In our study, there was no obvious association between MIS and maternal weight.

However, a statistically significant correlation of 0.22 was observed between MIS and maternal serum PAPP-A. This marker of Down's syndrome is produced by the syncytiotrophoblast, and in normal pregnancies, production increases with gestation. Thus, the observed association is consistent with a pregnancy-related increase in MIS because of placental secretion. Although there is no direct evidence for MIS

secretion into the blood by non-gonadal tissues, it may be relevant that the human MIS gene was cloned from a human placental library (Cate *et al.*, 1986). In contrast, at most, there appears to be a very small transient increase in MIS during pregnancy and this could be secondary to a hormonal modification of ovarian secretion.

A negative association was observed between MIS levels and maternal age. Such an association has also been found in several series of non-pregnant women who were naturally cycling or infertile and undergoing IVF (La Marca *et al.*, 2005b; Tremellen *et al.*, 2005; van Rooij *et al.*, 2002, 2004, 2005). The correlation coefficient was smaller in our series possibly due to the narrow maternal age range.

We examined maternal serum MIS levels in Down's syndrome pregnancies because of the biochemical and epidemiological evidence of diminished maternal ovarian reserve previously reported to be associated with aneuploidy. The fact that MIS levels were unaltered does not support a simple causal relationship between ovarian reserve and Down's syndrome. Warburton (2005), in a comprehensive review of biological ageing in the aetiology of aneuploidy, also concludes that ageing processes other than those measured by ovarian reserve and hormone levels are involved.

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