First trimester maternal serum concentrations of fetal antigen 2 in normal pregnancies and those affected by trisomy 21

K.M.Price1,4, J.M.M.Van Lith2, R.Silman1, A.Mantingh3 and J.G.Grudzinskas1

1Department of Obstetrics and Gynaecology, St Bartholomew’s & the Royal London School of Medicine and Dentistry, Queen Mary & Westfield College, London E1 1BB, UK, 2Department of Obstetrics and Gynaecology, Onze Lieve Vrouwe and Academic Medical Centre, Amsterdam and 3Department of Obstetrics and Gynaecology, University Hospital, Groningen, The Netherlands

4To whom correspondence should be addressed

Serum concentrations of fetal antigen 2 (FA-2), the aminopropeptide of the α1 chain of collagen type I, were measured in peripheral blood from women with normal (n = 234) and trisomy 21 affected (n = 14) pregnancies between 9 and 11 weeks gestation. Serum FA-2 concentrations were seen to be stable throughout this period, and though raised FA-2 concentrations were seen at the 10th week of gestation, a statistically significant difference between normal and trisomy 21 affected pregnancies was not found overall. Therefore it seems unlikely that FA-2 has a role in first trimester screening for trisomy 21, despite the fact that significantly higher FA-2 concentrations in trisomy 21 and significantly lower concentrations in trisomy 18 had been previously demonstrated in amniotic fluid in the second trimester.

Key words: collagen propeptide/fetal antigen 2/trisomy 21

Introduction

Fetal antigen 2 (FA-2) is a human protein, present in high concentrations in amniotic fluid, and shown to be identical to the aminopropeptide of the α1 chain of collagen type I (commonly abbreviated to PINP; Teisner et al., 1992). The presence of high concentrations of this molecule in the fetus is not surprising. Vanhaesebroeck et al. (1994) measured the related collagen type III propeptide in amniotic fluid and cord blood, and showed that concentrations reflected fetal growth activity. The existence of several molecular forms of PNIP has also been demonstrated (e.g. Tahtela et al., 1997). FA-2 molecular forms vary with size (Fay et al., 1988; Rasmussen et al., 1992) and/or charge (Price et al., 1994a) differences. We have described a radioimmunoassay for FA-2 (Price et al., 1994b) which is specific for high molecular mass FA-2 types (Price et al., 1995a). When applied to the comparison of FA-2 concentrations in amniotic fluid from normal pregnancies and pregnancies affected by trisomy, significantly higher FA-2 concentrations in trisomy 21 and significantly lower concentrations in trisomy 18 were seen (Price et al., 1995b).

The aim of the present study is to investigate the potential value of FA-2 as a first trimester serum screening marker for trisomy 21. There were two reasons why the study investigated the potential of FA-2 as a first trimester screening marker and not as a second trimester marker. Firstly, the current trend in screening for Down’s syndrome is to provide earlier diagnosis. This would offer many advantages to both clinicians and patients. Secondly, there are already many good second trimester markers, including human chorionic gonadotrophin (HCG), unconjugated oestriol and inhibin A. We believe that FA-2 is unlikely to outperform these markers in the light of the work of Puistola et al. (1993). Although they examined concentrations of the carboxy terminal peptide of procollagen type I and not the amino terminal peptide (identical to FA-2), both reflect the same process, i.e. collagen type I synthesis. Puistola et al. (1993) observed a wide range of maternal propeptide concentrations which did not rise above the normal non-pregnant range until late in the third trimester.

We have developed a more sensitive assay and applied it to the measurement of serum FA-2 concentrations in the peripheral blood from women with normal pregnancies (n = 234) and those affected by trisomy 21 (n = 14).

Materials and methods

Serum

The study was performed retrospectively on stored serum samples which had undergone up to four freeze–thaw cycles for previous analyses. It was considered unlikely that these storage conditions would have detrimentally affected FA-2 on the basis of previous work showing no significant changes in charge, molecular size, and antibody binding properties after 10 freeze–thaw cycles (Price et al., 1995a). Serum from 234 women with karyotypically normal pregnancies, and 14 women whose pregnancies were affected by Down’s syndrome, between 9 and 11 weeks gestation, were studied. All sera were collected from one hospital. Gestational age was determined from the first day of the last menstrual period and confirmed by ultrasound (crown–rump length) measurement. Karyotyping was performed by chorionic villus sampling immediately after serum collection. Serum was separated within 2 h of collection, and stored at –20°C.

Antibodies

Polyclonal antibodies were raised in rabbits against purified FA-2 as described by Price et al. (1994b).

FA-2 quantification

FA-2 was measured using a radioimmunoassay as described by Price et al. (1994b), except that antibody and standard (or sample) were preincubated for 24 h prior to addition of iodinated FA-2 tracer. Pooled second trimester amniotic fluid was used for calibration. FA-2 concentrations were expressed in µg/l based on a calibrator
Serum fetal antigen 2 in trisomy 21

Table I. Fetal antigen 2 concentrations in serum from women with normal pregnancies

<table>
<thead>
<tr>
<th>Week</th>
<th>n</th>
<th>Median concentration (95% CL) µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>109</td>
<td>48.5 (46.3–51.8)</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
<td>41.2 (38.9–46.3)</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>40.1 (37.9–45.8)</td>
</tr>
</tbody>
</table>

CL = confidence limit.

FA-2 concentrations throughout the first trimester of pregnancy

Serum samples (n = 234, normal karyotype) were quantified for FA-2. Median concentrations and 95% confidence intervals for each gestational week were determined (Table I). No statistically significant differences were seen in FA-2 concentrations throughout the period studied.

Comparison between FA-2 concentrations in normal and trisomy 21 serum in relation to gestation

Serum samples from 14 pregnancies affected by trisomy 21 were quantified for FA-2. FA-2 concentrations were plotted against weeks gestation and are presented in relation to the normal range (Figure 1). Though it can be seen that two out of four samples at 10 weeks gestation were above the normal range, no significant difference was found in median MoM values between trisomy 21 samples and the normal population overall (Table II).

Discussion

FA-2 in normal pregnancy

We have determined serum FA-2 concentrations from 234 women with normal pregnancies from 9 to 11 weeks gestation. A significant change in FA-2 concentrations was not seen throughout this time. There is little published information on serum concentrations of FA-2 or the aminopropeptide of collagen type I (PINP) during pregnancy. Our previous data showed low concentrations in the first and second trimester, and slightly higher concentrations in the third trimester (Price, 1995). Puistola et al. (1993) demonstrated a similar rise towards term in blood concentrations of the aminopropeptide of collagen type III (PIIINP), and the carboxypropeptide of collagen type I (PICP). This late rise contrasts with observations in amniotic fluid, where FA-2 concentrations rise steeply during the first trimester, peak during the second trimester, and fall towards term (Fay et al., 1988; Price et al., 1995b).

Previous studies have provided evidence for the fetal origin of FA-2 found in amniotic fluid (Fay et al., 1988; Tornehave et al., 1989). However, the origin of serum FA-2 is not clear. The median concentrations measured in the present study are within the normal reference interval for PINP (median: 56 µg/l; 10th and 90th percentiles: 30 and 82 µg/l) determined by Orum et al. (1996). This reference interval was defined.
using a different assay and calibrator, and therefore is not directly comparable with our data. However, Puistola et al. (1993) also found median concentrations of the related PIIINP and PICP peptides only rising above the non-pregnant reference interval after 30 and 34 weeks gestation respectively. Thus, it is likely that serum FA-2 concentrations in the first trimester are very similar to non-pregnant concentrations, and reflect collagen type I synthesis in maternal tissues unrelated to pregnancy.

**FA-2 in pregnancies affected by trisomy**

Median MoM serum FA-2 concentrations for trisomy 21 affected pregnancies were not significantly different from those seen in the normal population. The majority of trisomy 21 samples studied were at 9 weeks gestation. The data clearly demonstrate that FA-2 measurements do not discriminate between normal and affected pregnancies in contrast to markers such as β-human chorionic gonadotrophin (βhCG) and pregnancy-associated plasma protein-A (PAPP-A) at this stage of pregnancy (Van Lith, 1996). Although it is interesting to note that two of the four trisomy 21 samples at 10 weeks gestation were above the 90th percentile of the normal range, it seems unlikely that FA-2 has any role as a first trimester screening marker and the results do not justify a comprehensive prospective study.

**Acknowledgements**

This work was supported by a grant from the Medical Research Council (grant number: 9524393).

**References**


*Received on November 6, 1997; accepted on March 26, 1998*