

Maternal Serum S100 Protein in Normal and Down Syndrome Pregnancies

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Protein S100 is a low molecular weight (10–12 kD) calcium-binding protein the beta subunit of which is coded for at the 22.2–22.3 region of the long arm of chromosome 21. This region has also been shown to be responsible for the phenotypic expression of Down syndrome. Previous studies demonstrated increased immunoreactivity to protein S100 in brain tissue from adults with Down syndrome. We have previously observed a higher concentration of S100 protein in the fetal blood of trisomy 21 fetuses compared with normal subjects. The aim of this study was therefore to investigate the use of measuring S100 protein concentration in maternal blood for Down syndrome screening.

Maternal blood was taken at the time of chorionic villus sampling or cordocentesis (11–38 weeks' gestation) for fetal karyotyping. Protein S100 was measured by a two-site immunoradiometric assay (S-100 IRMA, Sangtec). There was no significant difference in the concentration of maternal S100 protein between normal and trisomy 21 pregnancies ($p < 0.10$). Moreover, there was no significant association between maternal serum S100 protein concentration and gestational age ($r_s = 0.27$, $p = 0.07$), maternal age ($r_s = -0.17$, $p = 0.7$) or maternal weight ($r_s = -0.013$, $p = 0.9$).

This study shows that measurement of maternal serum S100 protein concentration does not appear to have a value in Down syndrome screening. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS: S100 protein; Down syndrome; trisomy 21

INTRODUCTION

S100 protein is a low molecular weight (10–12 kD) calcium-binding protein, consisting of two subunits alpha (α) and beta (β) and is widely distributed in various tissues (Kilgman and Hilt, 1988). In humans high concentrations of S100 protein are found in glial cells in the brain, mainly of the β subunit (Isobe *et al.*, 1983). S100 protein is also found in neural and non-neural cell types either as the α subunit in muscle, the β subunit in Schwann cells and melanocytes, or as both subunits in chondrocytes and in the kidney (Haimoto *et al.*, 1987). The function of S100 protein is not fully known, however it has been shown that the protein is involved in the regulation of cell growth in human melanoma and rat glioma cells (Marks and Allore, 1990). Raised serum S100 protein concentrations have been found in patients with malignant melanoma and the concentration was related to disease progression (Abraha *et al.*, 1997a).

Several studies have shown that S100 protein is coded for at the 22.2–22.3 region of the long arm of chromosome 21 (Allore *et al.*, 1988; Marks and Allore, 1990; Korenberg *et al.*, 1990). This region has also been shown to be responsible for the phenotypic expression of Down syndrome (Park *et al.*, 1987). Previously an immunohistochemical study on human fetal brain tissue demonstrated increased expression of S100 protein in trisomy 21 compared with normal fetuses (Zuckerman *et al.*, 1970). The increased expres-

sion of S100 protein in fetuses with Down syndrome results in higher S100 protein concentrations in the fetal circulation (Abraha *et al.*, 1996), but it is not known whether the S100 protein can cross into the maternal circulation. The aim of this study was therefore to investigate whether measuring maternal serum S100 protein concentration could be useful in screening for Down syndrome.

MATERIALS AND METHODS

Serum S100 protein was measured in maternal serum from 117 pregnancies with normal fetuses and 22 with trisomy 21 fetuses. The maternal blood was obtained prior to chorionic villus sampling or cordocentesis at 11–38 weeks of gestation, performed for fetal karyotyping indicated by fetal ultrasound markers. In all cases the mother had given written consent for participation in the study. The study was approved by the Kings Healthcare Research Ethics Committee.

Blood was taken into a tube without anticoagulant and spun down at 1500 g within two hours of collection. The serum was stored at -20°C until analysis. Serum S100 protein was measured by an immunoradiometric assay (IRMA Sangtec, Bromma, Sweden). The antibody recognizes the beta subunit of S100 protein and requires 100 μl of serum. The assay was modified to give a lower standard of 0.3 $\mu\text{g/l}$ by diluting the highest standard (60 $\mu\text{g/l}$) with the diluent provided with the kit. The limit of detection was 0.05 $\mu\text{g/l}$ based on the point where the intra-assay imprecision exceeds a coefficient of variation (CV) of

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Table 1—Maternal serum S100 protein concentration in normal and trisomy 21 pregnancies and in non-pregnant women. Results are reported as median (range)

Maternal	Normal	Trisomy 21	Non-pregnant women
<i>n</i>	117	22	23
Gestation (weeks)	20 (11–38)	21 (12–38)	N/A
Age (years)	33 (16–46)	34 (17–41)	29 (22–49)
Weight (kg)	62 (47–109)	66 (47–98)	N/A
S100 ($\mu\text{g/l}$)	0.11 (0.07–0.36)	0.14 (0.07–0.28)	0.08 (0–0.15)

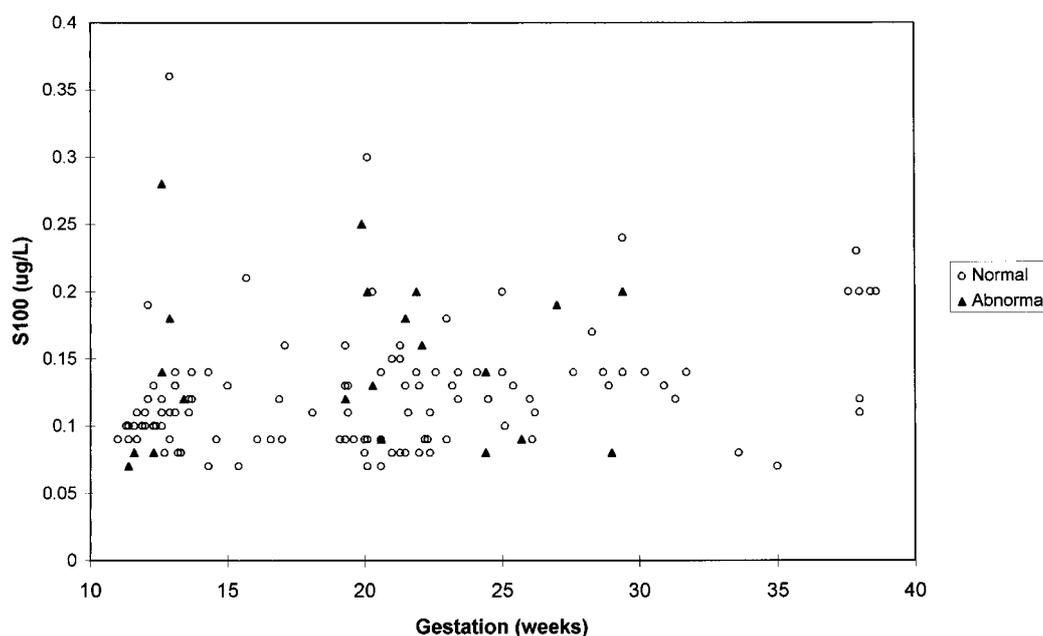


Fig. 1—Maternal serum S100 protein concentration in 117 normal and 22 trisomy 21 pregnancies

10 per cent. Intra-assay CVs were 2.8 per cent at $0.18 \mu\text{g/l}$ ($n=10$), 0.7 per cent at $1.5 \mu\text{g/l}$ ($n=10$) and 0.8 per cent at $5.3 \mu\text{g/l}$ ($n=10$) and inter-assay CVs were 3.3 per cent, 4.4 per cent and 1.5 per cent, respectively.

Data are reported as median and range for maternal age, gestational age, maternal weight and serum S100 protein concentration. Spearman's Rank Order Correlation coefficient was used for assessing associations between maternal serum S100 protein concentration, maternal age, gestational age and maternal weight. Statistical differences between normal and abnormal pregnancies were analysed using the Wilcoxon Mann–Whitney Test. Values of $p < 0.05$ were accepted as statistically significant.

RESULTS

The median and range of serum S100 protein concentration and the demographic data of the normal and trisomy 21 pregnancies are shown in Table 1. Data

from normal non-pregnant women are included in the table for comparison. There was no significant difference in maternal age or gestational age between the normal and trisomy 21 pregnancies. Although there was a trend towards higher maternal serum S100 concentrations in trisomy 21 affected pregnancies (Fig. 1) this did not reach statistical significance.

The maternal serum S100 concentration did not change significantly with gestational age ($r_s=0.27$, $p=0.07$), maternal age ($r_s=-0.17$, $p=0.7$) or maternal weight ($r_s=-0.01$, $p=0.9$).

DISCUSSION

Current strategies for Down syndrome screening are based on assay of biochemical markers in maternal serum (β -hCG, AFP, oestriol) with or without ultrasound. In the first trimester the combination of fetal nuchal translucency and maternal serum free

β -hCG can achieve a sensitivity of 85 per cent with a false-positive rate of 5 per cent (Noble *et al.*, 1996).

Theoretically, the presence of an additional copy of chromosome 21 in Down syndrome should result in increased concentrations of the gene products from any of the genes located on the chromosome. As yet, relatively few studies have been carried out to determine if this can be exploited for screening purposes. Miller *et al.* (1992) used the polymerase chain reaction to detect trisomy 21 in maternal blood using a specific primer of a region of the amyloid precursor protein (APP), localized on chromosome 21 and two other primers to the cystic fibrosis gene (CF), on chromosome 7. A raised ratio of APP/CF was found in Down syndrome cases compared with the control group and it was suggested that this was due to the expected increased gene dosage seen in trisomy 21. This was a preliminary study and further investigation is needed to determine if it is of any value in screening for Down syndrome (de Hann and Kola, 1994).

Several immunochemical studies have demonstrated increased expression of the β subunit of S100 protein in fetal tissues from trisomy 21 fetuses (Zuckerman *et al.*, 1970; Aita *et al.*, 1991; Mito and Becker, 1993). We have previously shown that the serum concentration of S100 in normal fetuses is approximately five-fold higher than that found in adults, i.e. 1.0–3.0 $\mu\text{g/l}$ (Abraha *et al.*, 1996). The serum concentration of S100 in the blood from trisomy 21 fetuses was higher than in unaffected fetuses by a factor of 1.5–2.0-fold, as expected from the gene dosage. In the present study maternal serum S100 protein was measured in normal and trisomy 21 pregnancies; there was no significant difference between the concentration of serum S100 protein in the two groups. Previously, Anneren *et al.* (1988) reported that the concentration of S100 protein measured in amniotic fluid in mid-trimester pregnancy was raised in only one case out of eight with trisomy 21 examined. This particular sample was also heavily blood stained, a factor itself shown in the study to be associated with increased S100 concentration.

Although the S100 concentration in fetal serum increased with gestational age, no relationship was found between maternal serum S100 concentration and gestational age. The serum S100 protein concentration found in pregnant women, both with normal and trisomy 21 affected fetuses, was similar to that found in non-pregnant women. In studies on serum S100 protein in malignant melanoma patients or following strokes we have not observed any difference in serum S100 concentration with gender or age in either normal subjects or affected patients (Abraha *et al.*, 1997a,b). A recent report has confirmed that serum S100 concentrations are age and sex-independent (Weismann *et al.*, 1998).

In patients with significant neurological damage from strokes or traumatic head injuries S100 protein is released into the cerebro-spinal fluid (CSF) and a small proportion leaks into the circulation (Abraha *et al.*,

1997b). The blood–brain barrier, however, maintains a concentration gradient that can be as high as 10:1 (CSF:serum). In the same way, the results from this study suggest that S100 protein does not cross the placenta and enter the maternal circulation. Maternal serum S100 concentration measurement is therefore not of value in Down syndrome screening.

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