Maternal serum placental growth factor in second trimester trisomy 21 pregnancies

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ORIGINAL ARTICLE

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ABSTRACT

Objective To investigate the levels of placental growth factor (PlGF) in second trimester maternal serum in trisomy 21 cases and euploid controls – an unclear subject in the published literature.

Methods Second trimester maternal serum samples from 17 recent (since 2005) and 74 older trisomy 21 cases and 542 euploid controls were extracted from frozen storage and retrospectively analysed for PlGF using a DELFIA Xpress immunoassay platform. Results were converted to multiples of median (MoM) for comparison.

Results The control group had a PlGF MoM of 0.99, the recent trisomy 21 cases had a PlGF MoM of 1.13 and the older cases a PlGF MoM of 1.11; however, the differences between trisomy 21 cases and controls were not significant.

Conclusion Although we have found no significant change in the second trimester levels of PlGF in trisomy 21 pregnancies, there remains wide disagreement within the literature on the behaviour of this marker during pregnancies of this syndrome. © 2012 John Wiley & Sons, Ltd.

INTRODUCTION

Placental growth factor (PIGF) is a dimeric glycoprotein member of the vascular endothelial growth factor (VEGF) family.1-3 PIGF is expressed in large amounts in the placenta, and, together with VEGF and their receptors, mediates angiogenesis in pregnancy4 and cell permeability.5 Pregnancies that go on to develop adverse pregnancy outcomes such as pre-eclampsia, or delivery of a small-for-gestational age fetuses, have been shown to have decreased detectable levels of PIGF in the first trimester of pregnancy.6-9

Chromosomally abnormal placentas often secrete, or transfer to the maternal circulation, aberrant levels of placental products, such as the markers pregnancy-associated plasma protein A (PAPP-A) and free-β-human chorionic gonadotrophin (free-β-hCG). Thus, PIGF has been investigated in pregnancies affected by trisomies of chromosome 13, 18 and 21 (T13, T18 and T21). In T13 and T18 pregnancies, a decrease in PIGF in the first trimester has been found compared with euploid controls in the two studies that have investigated it.10,11 More studies have been carried out exploring PIGF levels in T21, although there is little consensus amongst the results. In the first trimester, PIGF levels have been reported as increased10,12 and decreased7,11,13 in T21 cases when compared with controls. In the second trimester, also, PIGF levels have been found both increased,14 and decreased15 in T21 cases, and one paper reporting no difference between T21 cases and controls.16 Because of the likely future role for PIGF as a screening marker for pre-eclampsia, any part this marker may play in chromosomal anomaly screening requires clarification.

Screening for chromosomal anomalies is now common and continues to advance into farther parts of the world. First trimester screening that combines nuchal translucency (NT) and the biomarkers PAPP-A and free-β-hCG has increased in popularity because it offers detection rates as high as 92% at a 5% false positive rate.17 However, second trimester screening is still standard in some centres, and in the case of late presentation, it is the only option available.

We have recently examined PIGF levels in T21 cases and euploid controls in two first trimester intervals: early first trimester, weeks 8 to 10+612 and the first trimester screening window of weeks 11 to 13+7.7 Examining the data together, at around 8 to 9 weeks gestation, we found a small potential
increase in PlGF levels in T21 cases when compared with controls, although this seemed to normalise around 10 weeks. After which, around 12 to 14 weeks, levels in T21 cases appeared to decrease slightly compared with controls. The current study attempts to further the discussion, by examining PlGF levels in T21 cases and controls in the second trimester screening window of 14 to 20 weeks using the DELFIA Xpress PlGF assay.

METHODS

Study population
This was a retrospective case-control study of second trimester pregnancies screened at our clinic. The majority of our patients present in the first trimester, so the number of T21 cases identified during the second trimester is low. Freezer capacity limits the storage of control samples to 5 years, so the sample set consists of (i) 17 recent T21 cases screened between April 2005 and October 2009, for which 167 euploid controls matched on storage time (±1 day) and gestational age (±1 day) and (ii) 74 older T21 cases screened between July 1993 and December 2004, for which 375 euploid controls were selected, approximately 50 per completed gestational week (14–20 weeks). In total there were 91 T21 cases and 542 euploid controls.

Serum alpha-feto protein (AFP) and free-β-hCG concentrations were determined at the time of screening, prior to June 1998 by Immunoradiometric assays (IRMAs) previously described and, since June 1998 by the Kryptor analyser (Thermo Fisher Scientific, Waltham, MA, USA). Pregnant women had given informed consent for surplus blood to be used for research, approved by our Institutional Review Board. Serum was stored at −20°C. Pregnancies were dated by biparietal diameter measurements. Maternal characteristics were recorded at the time of screening and all data were stored on a local database. Diagnostic results were added to the database after being received from the cytogenetic laboratory or maternity unit in which the delivery occurred.

PIGF quantification
Placental growth factor concentration was determined by using a solid phase, two-site fluoro-immunometric assay on the 6000 DELFIA Xpress random access platform (PerkinElmer, Turku, Finland). This is an automated assay in which 40 μL of either sample, calibrator or quality control is pipetted into a cup, which is pre-coated with anti-PIGF rabbit polyclonal capture antibody. This is followed by the addition of 35 μL of assay buffer and 5 μL tracer (europium labelled anti-PIGF mouse monoclonal antibody). Protein–antibody complexes are left to form during 20 min of incubation at 35°C. The cup is then washed, 100 μL of DELFIA Inducer is added and the fluorescence is measured relative to PlGF concentration compared with a calibration curve constructed at the beginning of the kit lot. Samples were run in triplicate and the mean value was used.

Smoking status
The majority of our samples had unknown maternal smoking status because of the fact that self declared smoking status has only been routinely recorded in the database for second trimester screening since 2008. We knew that in the first trimester, smoking had a significant effect on maternal serum PlGF levels. Maternal serum cotinine levels were determined using a manual serum enzyme-linked immunosorbent assay kit (Cozart, Abbingdon, UK). In a 96-well plate, precoated with anti-cotinine capture antibody, 10 μL of sample, calibrator or quality control were added to a well. A solution of 100 μL containing the enzyme-conjugate was then added to each well and the plate was incubated for 30 min. Following a wash step, 100 μL substrate solution was added and stopped with 100 μL 1 mol/L sulphuric acid after 30 min incubation. Absorbance was read at 450 nm in a Victor spectrofluorometer (PerkinElmer, Turku, Finland), and sample values were compared with a standard curve, which included an additional low standard at 5 ng/mL, which was constructed by diluting the 10 ng/mL standard 1:2 with the kit 0 calibrator. Although the kit instructions suggested a value of 25 ng/mL to differentiate smokers from nonsmokers, evidence from the literature suggests a value closer to 15 ng/mL to distinguish between each group. We therefore used a cut-off of 15 ng/mL of cotinine to distinguish between smokers and nonsmokers.

Data analysis
Multiple regression was applied to the data from the euploid control samples to assess the effects of gestational age, smoking status, maternal ethnicity and maternal weight on PlGF levels. PlGF concentrations were then corrected for these factors by conversion to MoM using the expected value obtained from this regression.

The Mann–Whitney U-test was used to compare the levels of PlGF MoMs in the control and trisomy 21 cases groups. The Pearson product moment correlation analysis was carried out to determine whether log10 PlGF MoMs correlated with log10 AFP and log10 free-β-hCG MoMs.

Data were analysed using R (GNU project http://cran.r-project.org).

RESULTS

Maternal demographic data are summarised in Table 1. Maternal age was higher in the T21 groups than the controls, as anticipated. The gestational age was also different between the T21 groups and the controls because of the way the controls were selected. There were significantly fewer smokers in the T21 groups, presumably because of the inverse association with pregnancy smoking and maternal age.

In the control pregnancies, PlGF levels increased with gestational age, shown in Figure 1. Multiple regression of the control group showed that gestational age, maternal smoking status, maternal weight and maternal ethnicity were significant independent contributors to log10 PlGF. The following equation

was used to calculate the expected PlGF concentration for each sample:

\[
\text{Expected \ Log10\ PlGF} = 0.381 + 0.0130 \times \text{gestational age (days)} \\
+ 0.113 \text{ [if Asian]} + 0.264 \text{ [if Black]} + 0.0980 \text{ [if other]} \\
+ 0.145 \text{ [if smoker]} - 0.00157 \text{ [maternal weight (kg)]}
\]

The median MoM in the control group was 0.99. In the trisomy 21 cases collected before 2005, the median MoM was 1.11, and in the trisomy 21 cases collected after 2005, the median MoM was 1.13. However, there were no statistically significant differences between the case groups and the controls (\(p = 0.90\) and \(p = 0.77\), respectively).

In the trisomy 21 cases, gestational age did not correlate with log10 PlGF MoM (\(r = -0.01\), \(p = 0.93\)). In the euploid group, log10 PlGF correlated with log10 AFP with a coefficient \(r = 0.15\) (\(p < 0.01\)) and correlated with log10 free-\(\beta\)-hCG with a coefficient \(r = 0.12\) (\(p = 0.01\)).

**DISCUSSION**

Although this study has found slightly higher maternal serum PlGF MoM in trisomy 21 cases than in euploid controls, the differences were not significant.

Placental growth factor is an important mediator of angiogenesis and is expressed in large amounts in the placenta. Its biological activity is attributed to binding VEGF-Receptor 1, also known as fms-like tyrosine kinase 1 (Flt-1), either as a PlGF homodimer, where it is thought to potentiate the effects of angiogenic effects of VEGF, or as a VEGF/PlGF heterodimer.\(^4\),\(^24\) sFlt-1 is a soluble form of the receptor, and acts as a natural competitive inhibitor of PlGF and VEGF, resulting in a decrease in their biological activity. A proportion of PlGF in the circulation is bound to sFlt-1, and PlGF bound to sFlt-1 is undetectable by commercial PlGF assays, which only detects the free form.\(^25\),\(^26\)

Placental growth factor is almost undetectable in non-pregnant serum, and in normotensive euploid pregnancies, maternal serum PlGF levels rise with gestation, starting off low at 8 weeks’ gestation,\(^12\) and increasing through the rest of the first trimester\(^7\) and into the second trimester as shown in this study. Over the previous decade, maternal serum PlGF concentrations in the first and second trimesters of trisomy 21 pregnancies have been examined by several groups, in comparison with normal pregnancy levels. However, results have not always been in agreement with each other. In the first trimester, examining serum samples taken between 10 to 14 weeks, the initial study carried out found an increase in PlGF levels in trisomy 21 cases compared with controls.\(^10\) However, since this, studies looking at the same gestational age window have all reported a relative decrease in PlGF levels in trisomy 21 cases,\(^7\),\(^11\),\(^13\) although we most recently reported an increase in PlGF levels in trisomy 21 samples drawn before
10 weeks compared with controls. Previous studies examining the second trimester window paint a no clearer picture. Although one study has reported a decrease in PI GF at this screening window, another reported an increase, and a third, like us, was unable to find any significant differences in levels between the trisomy 21 and controls. Thus, the biochemical role of PI GF in trisomy 21 remains unclear. Debieve et al. suggested that the decreased levels of maternal serum PI GF they found in the second trimester in trisomy 21 pregnancies may contribute to the histopathological abnormalities observed in trisomy 21 placentas, including hypovascularity and trophoblast hypoplasia. They also suggested that the vascular permeability properties of PI GF may play a role in the normal transfer of markers such as AFP and unconjugated estriol across the placenta into the maternal circulation in normal pregnancies. In trisomy 21 pregnancies they proposed that a decrease in PI GF, and therefore in vascular permeability, leads to low levels of maternal serum levels of these markers found in trisomy 21 screening, rather than a decrease in production of these products by the fetus. 

Su et al., who, in the same gestational window found the exact opposite — a relative increase in PI GF levels in trisomy 21 pregnancies — postulated that some loci on chromosome 21 might interact with the gene for PI GF on chromosome 14, and that the additional copy of chromosome 21 may up-regulate the expression of PI GF or down-regulate its degradation. Despite finding small but significant differences in PI GF levels in trisomy 21 levels at 8 to 10 weeks and 11 to 13 weeks compared with controls, we were unable to find any significant differences in the second trimester in the current study. Although another paper has also failed to find changes at this gestational window, too many questions remain as to why such discrepancies exist in the literature around PI GF in trisomy 21 pregnancies. Although the current and other recent studies have used the DELFIA Xpress PI GF assay (PerkinElmer, Turku, Finland) to quantify the biomarker, studies prior to 2009 have used the Quantikine human PI GF assay (R&D Systems, Minneapolis, MI, USA); however, inconsistencies still exist between studies that used the same assay. Furthermore, not all studies have corrected for maternal factors, in particular smoking, which we have found to cause a significant increase in PI GF levels, which has been reported before. A final factor to consider is sample stability which, because of the age of some of our samples, may be a limitation in this study. We have recently reported that serum left at room temperature and above results in increased PI GF levels with time, presumably because of the break down of sFlt-1 bound PI GF releasing free and detectable PI GF. However, other papers have found that PI GF is not affected by frozen storage.

In conclusion, the behaviour of PI GF in trisomy pregnancies relative to euploid controls remains unclear, especially in the second trimester, because of the disagreement reported in the literature over the small number of retrospective studies available. If PI GF holds any value as a screening tool for trisomy 21, or other aneuploidies, it seems unlikely to be revealed using the methods currently available. However, if PI GF becomes routinely measured in large numbers of pregnant women for early screening for pre-eclampsia, as has been proposed, then data sets will soon be available where early gestation maternal serum PI GF levels of trisomy 21 and normotensive euploid pregnancies can soon be compared in large numbers, hopefully shedding more light on the subject.

**REFERENCES**


