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Maternal serum PlGF (placental growth factor) in Chinese women in the first trimester undergoing screening for Down syndrome

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ABSTRACT

Objective: To evaluate the potential of maternal serum placental growth factor (PlGF) as a marker for Trisomy 21 in Chinese pregnant women.
Study design: Serum samples were collected and stored from 600 women who have a viable singleton pregnancy and underwent first trimester screening for Trisomy 21 between 2011 and 2014. Serum concentration of PlGF was measured using the automated time-solved immunofluorometric assay and expressed as Multiples of the expected median (MoM) from the 600 women available stored serum samples (558 Euploidy and 42 Trisomy 21). The levels of PlGF MoM were compared between Trisomy 21 cases and Euploid pregnancies. Expected median PlGF levels in Chinese were also compared to that published for Caucasians. Multivariate Gaussian modeling was performed to predict detection and false-positive rates.
Results: In euploid pregnancies the concentrations of PlGF increased with Crown Rump Length(CRL) and decreased with maternal weight. The overall median MoM of PlGF in Chinese was higher than that of Caucasian. The median PlGF level was 0.63 MoM in the cases and 1.00 MoM in the controls ($p < 0.0001$). The prediction of Trisomy 21 has been slightly improved by the addition of PlGF to the standard screening test in China. The detection rate of screening after adding PlGF data was increased from 93.4% to 94.6% at a false-positive rate of 3% and the false positive rate decreased from 0.23% to 0.17% for a detection rate of 80%.
Conclusion: The median PlGF concentrations in Chinese is higher than those of Caucasians. Results suggest adding PlGF may substantially improve the performance of current first trimester screening strategy for Trisomy 21 in Chinese pregnant women.

Introduction

Placenta Growth Factor (PlGF) is derived from the placenta as a secreted growth factor for vascular endothelial cells and based on its homology to vascular endothelial growth factor (VEGF), which can be classified as a member of platelet derived growth factor (PDGF) family [1]. PlGF is believed to control both placental angiogenesis and the trophoblastic invasion of maternal spiral arteries [2]. Reduced levels of PlGF in maternal serum during pregnancy have been reported to be associated with the development of preeclampsia [3–5], small for gestational age (SGA) births [6,7], as well as a marker for aneuploidy [8].

The studies performed by Zaragoza et al. [9] and Pandya et al. [10] indicated that PlGF was a potentially useful discriminator between unaffected and Trisomy 21 in early pregnancy. Modeling studies by the same group indicated that its inclusion in first trimester combined screening would increase the detection rate for Trisomy 21 from 85% to 88% and reduced the false positive rate from 2.7% to 2.6% at a cut-off of 1 in 100. But these two published studies on the level of PlGF have been based on populations in which subjects have been predominantly 'Caucasian' (European) in their ethnic origin, other ethnicities were reported in a small number or short of Trisomy 21 cases. The maternal pregnancy characteristics have been shown to be important factors in the

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level of first trimester markers such as free beta HCG (free β-hCG) and pregnancy-associated plasma protein A (PAPP-A) [11,12]. Kagan et al. [13] reported that Afro-Caribbeans had higher levels compared to Caucasians after adjusting for covariates, a finding similar to that reported for PAPP-A and free β-hCG. East Asians, and specifically Chinese, have also been reported to have higher or lower median levels of PAPP-A and free β-hCG after adjusting for covariates. We also previously proved the marker ADAM12 (A Disintegrin and Metalloprotease 12) concentrations in Chinese are lower than those of Caucasians and Afro-Carribeans [14]. The aims of the study were firstly to report the gestational median levels of maternal serum PlGF in Chinese; secondly determine whether adding the PlGF can improve the performance of first trimester combined screening for Down syndrome in Chinese pregnant women.

Materials and methods

Study population

This was a case–control study on serum samples collected from Chinese women who have an ultrasound confirmed viable singleton pregnancy of known gestational age and came to Guangzhou Women and Children's Medical Center for routine first trimester screening for aneuploidy between 2011 and 2014. During the hospital visit, which was held at 11–13^{+6} weeks, the ultrasound examinations were firstly performed by doctors with experience in obstetric ultrasound and certified by The Fetal Medicine Foundation (FMF) in London, UK. Fetal crown rump length (CRL) and nuchal translucency (NT) thickness were measured using standardized techniques as described by the Fetal Medicine Foundation (FMF) (www.fetalmedicine.com). The fetal structures were also examined to diagnose the major fetal structural defects. After ultrasound scan, blood was taken at the same day and tested for serum PAPP-A and free β-hCG levels using commercially available kits (AutoDelfia Xpress system, PerkinElmer life and Analytic Science, USA). In all cases, the software of lifecycle 3.2 (PerkinElmer Life science, Turku, Finland) was used to calculate the patient-specific risk of fetal Trisomy 21 as well as to derive PAPP-A and free β-hCG MoMs adjusted for maternal weight, ethnicity, mode of conception and smoking status. Women were given their estimated individual risk for Down syndrome and Trisomy 18, and those who were considered high risk taken the invasive procedures such as chorionic villus sampling or amniocentesis for fetal karyotyping. The outcomes of pregnancies were followed after six months delivery. All information was recorded in a clinical audit database for subsequent analysis.

Sample analysis

After having obtained informed consent from the patients, the remaining serum samples were stored together with pregnancy information about the karyotype, maternal age, maternal weight, crown-rump length, gestational age at the blood sampling, smoking status and samples storage time. The serum samples were then stored at −20 °C for up to 24 h and then subsequently stored at -80 °C until being analyzed. The measurements of serum PlGF at 11–13^{+6} weeks were part of a research study which have been approved by the appropriate Guangzhou Women and Children's Medical Center's Ethics Committee.

The concentration of PlGF was measured using a single sample of 50 μl by an automated time-solved fluorescent immunoassay (Delfia/AutoDelfia PlGF Research Kit, PerkinElmer Life and Analytical Science, Turku, Finland). Fresh recombinant human PlGF quality control samples, with concentration ranging from 42 pg/ml to 123 pg/ml, where measured in duplicate at the beginning and end of each run. The mean coefficient of variations for the respective quality control samples were 4.8% for low concentration (42 pg/ml) and 3.7% for the high concentration (123 pg/ml) sample.

Statistical analysis

Maternal demographic characteristics of the cases and controls were compared using Wilcoxon test. Serum marker concentrations were converted to MoMs by weighted regression analysis to allow levels of serum markers change with gestation. The median level was regressed against the median gestational days in the group, weighted by the number of observations in the group. MoM values were then adjusted for maternal weight. Wilcoxon tests were performed to compare median PlGF MoMs in affected pregnancy and unaffected controls. $P < 0.05$ was considered statistically significant.

Model-predicted screening performance for combined test and combined test plus PlGF was estimated by multivariate Gaussian modeling with parameters derived directly from the cases and controls in current study. The medians were from the observed MoMs and the standard deviations (SDs) were estimated by the $\log_{10}$ 10th–90th percentile range divided by 2.563. After excluding outliers greater than 3SDs from median in the aneuploid and normal groups, Pearson correlation analysis was carried out to determine the correlation coefficients between $\log_{10}$ MoM of markers. Numerical integration [15] was used to sum risks over a modeled maternal age distribution by applying the log Gaussian distribution of markers to a standardized maternal age distribution [16] and a maternal age-specific birth prevalence curve derived from meta-analysis for a fixed false-positive rate or vice versa [17].

The selection of the specific serum samples from women screened for PlGF concentration measurement and subsequent statistical analysis was simply based on availability. Two-tailed *t*-test was used to compare if there are any differences in mean of maternal age at estimated due date (EDD), gestation at screening, and maternal weight between those with and without available samples. The investigators performing measurement of the PlGF concentration were blinded to chromosomal status of the pregnancy.

Results

During the study period 22,300 women underwent screening of which 42 (0.2%) were confirmed as having Trisomy 21 and 37 (0.16%) had other chromosomal abnormalities (Trisomy 13, 18, Triploidy, Turner's syndrome and other aneuploidies). Stored serum samples in pregnancies with a known outcome were available for measurement of PlGF concentration in 600 pregnancies, 42 of which had Trisomy 21 and 558 examined of being normal euploidy. There were no significant differences in the maternal age at EDD ($p < 0.01$), gestation at screening ($p < 0.05$) and maternal weight ($p < 0.01$) between those with and without available samples. Table 1 shows the characteristics for both Down syndrome and euploid cases. All reported that their pregnancy was naturally conceived, were non-smokers, and that they did not have insulin-dependent diabetes mellitus and none had a past history of having a pregnancy affected by chromosomal abnormality.

The numbers of unaffected pregnancies in the 11, 12 and 13 weeks were 141 (25.27%), 234 (41.93%) and 183 (32.8%), respectively. PlGF concentrations ranged from 12.0 to 243.6 pg/ml. The median concentrations of PlGF in euploidy in week 11, 12 and 13 were 35.9 pg/ml, 43.9 pg/ml, and 55.6 pg/ml, respectively. PlGF concentrations in the 42 pregnancies with Trisomy 21 ranged from 15.7 to 115.6 pg/ml. The median concentrations were 26.1, 27.9, and 36.1 pg/ml in weeks 11, 12 and 13, respectively. PlGF