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First trimester hyperglycosylated human chorionic gonadotrophin in serum – A marker of early-onset preeclampsia

E. Keikkala [a,*], P. Vuorela [b], H. Laivuori [a,c,d], J. Romppanen [e], S. Heinonen [f], U.-H. Stenman [g]

[a] Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Biomedicum Helsinki, PB 700, 00029 Helsinki, Finland
[b] Department of Obstetrics and Gynecology, Porvoo Hospital, PB 500, 06151 Porvoo, Finland
[c] Department of Medical Genetics, Haartman Institute, University of Helsinki, PB 63, 00014 Helsinki, Finland
[d] Women's Health, Research Programs Unit, University of Helsinki, 00014 Helsinki, Finland
[e] Eastern Finland Laboratory Centre, PB 1700, 70211 Kuopio, Finland
[f] Department of Obstetrics and Gynecology, Kuopio University Hospital and University of Eastern Finland, PB 1777, 70211 Kuopio, Finland
[g] Department of Clinical Chemistry, University of Helsinki, PB 700, 00029 Helsinki, Finland

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ABSTRACT

Introduction: Recent studies indicate that treatment with low-dose aspirin may reduce the risk of preeclampsia. Thus, early prediction of preeclampsia is needed. Low serum concentrations of hyperglycosylated human chorionic gonadotrophin (hCG-h) are associated with early pregnancy loss. We therefore studied whether it may serve as an early marker of preeclampsia.
Methods: A nested case-control study included 158 women with subsequent preeclampsia, 41 with gestational hypertension, 81 normotensive women giving birth to small-for-gestational-age (SGA) infants and 427 controls participating in first trimester screening for Down's syndrome between 8 and 13 weeks of gestation. Gestational-age-adjusted multiples of medians (MoMs) were calculated for serum concentrations of hCG-h, the free beta subunit of hCG (hCGβ) and pregnancy-associated plasma placental protein A (PAPP-A) and the proportion of hCG-h to hCG (%hCG-h). Clinical risk factors including mean arterial pressure (MAP) and parity were also included in the risk calculation.
Results: In women with subsequent preeclampsia %hCG-h was lower than in controls (median MoM 0.92, $P < 0.001$), especially in 29 cases with early-onset preeclampsia (0.86, $P < 0.001$), in which PAPP-A also was reduced (0.95, $P = 0.001$). At 90% specificity for prediction of early-onset preeclampsia, sensitivity was 56% (95% confidence interval, 52–61%) for %hCG-h, 33% (28–37%) for PAPP-A, and 69% (51–83%) for the combination of these with first trimester MAP and parity. The area under the receiver-operating characteristic (ROC) curve for the combination of all these was 0.863 (0.791–0.935).
Conclusions: hCG-h is a promising first trimester marker for early-onset preeclampsia. Addition of PAPP-A and maternal risk factors may improve the results.

1. Introduction

Preeclampsia affects approximately 3% of all pregnancies [1]. Its etiology is unknown but inadequate placental development is a typical feature [2]. Inadequate adaptation of uterine spiral arteries is thought to lead to an ischemia-reperfusion injury in the placenta [3–5]. Extravillous cytotrophoblasts fail to adopt an invasive phenotype [2], causing impaired invasion of these into the maternal spiral arteries and abnormal function [4,6]. Recently, low-dose aspirin started before 16 weeks of gestation has been shown to reduce the risk of developing preeclampsia [7]. Therefore, screening tests identifying women at risk are of great potential utility.

Determination of the free beta subunit of hCG (hCGβ) and pregnancy-associated plasma placental protein A (PAPP-A) is used in first trimester screening of Down's syndrome [8]. Increased serum concentrations of hCGβ are thought to reflect delayed placental maturation associated with Down's syndrome [9]. However, studies on the association of hCGβ with subsequent preeclampsia or small-for-gestational-age (SGA) infants have given conflicting results [10–12]. PAPP-A, expressed by the placenta, may contribute to placental and fetal growth [13]. Low first trimester

* Corresponding author. Tel.: +358 9 4717 17 28; fax: +358 9 4717 17 31.
E-mail addresses: elina.keikkala@hus.fi (E. Keikkala), piia.vuorela@hus.fi (P. Vuorela), hannele.laivuori@helsinki.fi (H. Laivuori), jarkko.romppanen@islab.fi (J. Romppanen), seppo.heinonen@kuh.fi (S. Heinonen), ulf-hakan.stenman@helsinki.fi (U.-H. Stenman).

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Abbreviations

hCG-h	hyperglycosylated human chorionic gonadotrophin
SGA	small-for-gestational-age
MoM	multiple of the median
hCGβ	the free beta subunit of hCG
PAPP-A	pregnancy-associated plasma protein A
hCG	human chorionic gonadotrophin
MAP	mean arterial pressure
ROC curve	receiver-operating characteristic curve
BMI	body mass index
ACOG	American College of Obstetricians and Gynecologists
HELLP	hemolysis, elevated liver-enzymes, and a low platelet count syndrome
CV	coefficients of variation
AUC	area under the ROC curve
CI	confidence interval
IQR	interquartile range
IL-10	interleukin-10
sFlt-1	soluble fms-like tyrosine kinase 1
sEng	soluble endoglin
PlGF	placental growth factor
PP13	placental protein 13

serum PAPP-A concentrations have been observed in pregnancies with subsequent preeclampsia [12] and SGA infants [10,11]. Clinical risk factors are often combined with biochemical markers to improve assessments of preeclampsia risk. Nulliparity, high body mass index (BMI) and elevated first trimester blood pressure are independent risk factors of preeclampsia, whereas smoking has been shown to reduce the risk [14–17]. Maternal age over 40, chronic diseases, e.g. type 1 diabetes and hypertension, are other risk factors [15].

Hyperglycosylated human chorionic gonadotrophin (hCG-h) is a variant of hCG with more complex glycan structures than hCG from mid and late pregnancy [18,19]. In early pregnancy, hCG-h is the major form of hCG in urine and serum [20]. Its proportion out of hCG decreases from 90 to 100% in early pregnancy to less than 3% at 20 weeks of gestation [21]. hCG-h is thought to enter maternal circulation mainly from the invading extravillous cytotrophoblasts and at lower levels from villous cytotrophoblasts, whereas regular hCG is produced mainly by placental syncytiotrophoblasts [22,23]. Low serum concentrations of hCG-h are associated with early pregnancy loss [20], and low mid-trimester hCG-h concentrations in urine have been associated with subsequent preeclampsia [24].

The aim of this case-control study was to determine whether first trimester serum concentrations of hCG-h, hCG, PAPP-A and hCGβ, either alone or combined with maternal clinical risk factors, can be used to identify women at risk of developing preeclampsia.

2. Methods

Blood samples were collected from 12,615 pregnant women attending combined first trimester screening for Down's syndrome at 8–13 weeks of gestation in Kuopio University Hospital region between April 1, 2008 and December 31, 2010. Gestational age was ascertained by ultrasound measurement of fetal crown-rump length. Women with multiple gestations and/or major fetal anomalies were excluded. The study was approved by the Ethical Research Committee of Kuopio University Hospital and all participants gave written informed consent.

Preeclampsia was defined according to the criteria of the American College of Obstetricians and Gynecologists (ACOG): a systolic blood pressure of 140 mm Hg or higher, or diastolic pressure of 90 mm Hg or higher occurring after 20 weeks of gestation in a woman with previously normal blood pressure in combination with a urinary 24-hour protein excretion of 0.3 g or more [25]. Women with superimposed preeclampsia, elevated blood pressure before 20th week of pregnancy or history of essential hypertension, were also included in the study [25]. Based on hospital records, preeclampsia was diagnosed in 273 pregnancies (2.16%). Eventually, 159 pregnancies with subsequent preeclampsia were eligible for the study (Fig. 1). Early-onset preeclampsia (onset before 34 weeks of gestation) was diagnosed in 29 pregnancies [26]. Thirty-six preeclamptic women delivered SGA infants as defined by an age- and sex-adjusted birth weight below the 10th percentile [27]. Another 81 normotensive women with SGA infants were included in the study. Two women had repeated pregnancies and both had preeclampsia in their first pregnancies, whereas one developed hypertension and the other early-onset preeclampsia in the second pregnancy. Thus, our study population comprised 158 Caucasian women with 159 preeclamptic pregnancies (Table 1).

We aimed to select three controls per case amongst women without preeclampsia, gestational hypertension, or SGA based on hospital records and delivered during the same time period. Thus, some controls may have had other pregnancy complications. Altogether 427 women, matched as closely as possible by gestational age at the sampling time, maternal age and BMI determined between 7 and 10 weeks of gestation were identified. For some cases with the highest BMIs we only found two controls since matching BMIs were rare in unaffected women (Fig. 1, Table 1).

Blood samples were allowed to clot at room temperature for 30 min, centrifuged and stored at +4 °C. Serum samples were delivered to the analytical laboratory cold or frozen and stored at −20 °C. Serum concentrations of hCG, hCGβ and PAPP-A were analyzed by time-resolved fluoroimmunoassay according to manufacturer's instructions (Perkin Elmer WallacOy, Turku, Finland). Remaining serum was stored at −20 °C until analysis of hCG and hCG-h in September 2011. The hCG assay measures heterodimeric hCG including hCG-h but not hCGβ. Concentrations of hCG-h were analyzed by a recently described time-resolved immunofluorometric assay [22]. The intra- and inter-assay coefficients of variation (CV) were <1.8% and <3.7% for PAPP-A, <2.3% and <4.1% for hCGβ, 1.8% (mean) and <8.8% for hCG, and 2.2% and <10.8% for hCG-h, respectively. CVs were determined in ten aliquots of two serum pools analyzed either in the same or consecutive runs. The calibrators covered the ranges 10–2000 mU/L for PAPP-A, 2–200 ng/mL for hCGβ, 5–30,600 pmol/L for hCG, and 9–9000 pmol/L for hCG-h. Serum samples were diluted 5-fold prior to assay of PAPP-A and 100-fold prior to assays of hCG and hCG-h. This eliminates interference by complement in the hCG-h assay [22].

2.1. Statistical analysis

Principal component regression analysis was used to adjust the concentrations of hCG-h, hCGβ and PAPP-A, and the proportion of hCG-h to hCG (%hCG-h) for gestational age and express the results for each case as multiples of the median (MoM). The regression equation for the medians of log hCG-h was 7.41−0.28 * gestational age (weeks), for log %hCG-h it was 2.85−0.71 * gestational age (weeks) and that for log PAPP-A 0.37 * gestational age (weeks) −0.94 and for log hCGβ 4.61−0.28* gestational age (weeks). According to the Kolmogorov–Smirnov's test, the concentrations of hCG, hCG-h, hCGβ, PAPP-A and %hCG-h were log-normally distributed while the MoM values were normally distributed. Differences between cases and controls were estimated by ANOVA and post hoc comparisons for controls by Dunnett's test. Clinical characteristics of the groups were compared by the Mann–Whitney *U* test for continuous variables or by the χ^2 test for dichotomized variables. Correlations between clinical characteristics and biomarker concentrations were analyzed by multivariate linear regression analysis. The contribution of different markers and maternal factors to the risk of preeclampsia was analyzed by logistic regression. The diagnostic accuracy was analyzed by

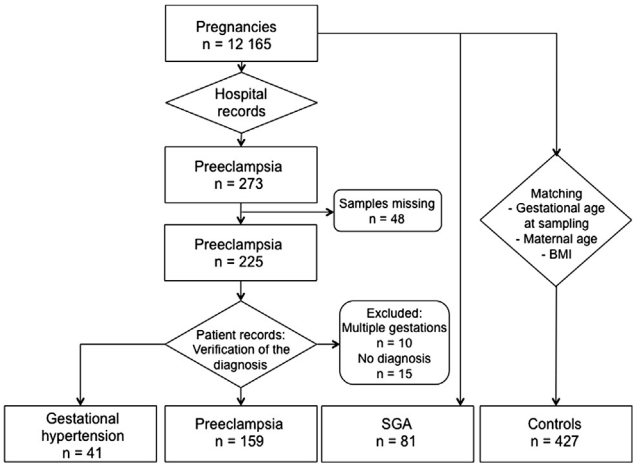

Fig. 1. Flow chart of patient selection.