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Human chorionic gonadotropin and risk of pre-eclampsia: prospective population-based cohort study

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KEYWORDS: angiogenesis; hCG; placenta; PlGF; pre-eclampsia; sFlt-1

CONTRIBUTION

What are the novel findings of this work?
Several studies have suggested that human chorionic gonadotropin (hCG) concentration is associated with pregnancy outcomes, including pre-eclampsia. However, the underlying mechanisms remain largely unexplained. This study shows an association of high total hCG with a high risk of pre-eclampsia, which could be explained in part by the effects of hCG on the balance between pro- and anti-angiogenic factors, placental growth factor and soluble fms-like tyrosine kinase-1.

What are the clinical implications of this work?
The results of this study, showing the association of high hCG concentration with risk of pre-eclampsia, could be incorporated into clinical prediction models. This means that, by using hCG concentration profile, together with other risk factors, the probability of adverse events in pregnancy, including the risk of developing pre-eclampsia, could be analyzed.

ABSTRACT

Objectives *Abnormal placentation in early pregnancy may play a role in the pathogenesis of pre-eclampsia. Human chorionic gonadotropin (hCG) regulates placental development and angiogenesis and may affect the ratio of soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) in the serum. The aims of this study were to investigate the association of total hCG with the risk of pre-eclampsia and to examine the potential effect of pro- and anti-angiogenic factors on this association.*

Methods *This was a population-based prospective cohort study of 7754 women with a singleton pregnancy. Total hCG was measured in the first available sample (median gestational age, 14.4 weeks; 95% range, 10.1–26.1 weeks) and sFlt-1 and PlGF concentrations in early (< 18 weeks; median, 13.2 weeks; 95% range, 9.6–17.6 weeks) and in mid- (18–25 weeks; median, 20.4 weeks; 95% range, 18.5–23.5 weeks) pregnancy. We tested the association of hCG concentration and risk of pre-eclampsia using regression analysis, adjusting for maternal age, ethnicity, body mass index, parity, education level, smoking status and fetal sex. Additionally, we assessed whether this association was affected by the sFlt-1/PlGF ratio.*

Results *High hCG concentration was associated with a 1.5–2.7-fold increased risk of pre-eclampsia (*$P = 0.0001$*), depending on the cut-off used, and with increased sFlt-1/PlGF ratio during early pregnancy (*$P < 0.0001$*). The association between high hCG and pre-eclampsia attenuated by roughly 40% after adjustment for early-pregnancy sFlt-1/PlGF ratio (β-estimate change from* 0.19 ± 0.10 *(*$P = 0.052$*) to* 0.12 ± 0.10 *(*$P = 0.22$*)).*

Conclusions *High total hCG concentration in early pregnancy is associated with an increased risk of pre-eclampsia. The effect of high hCG concentration on the balance between pro- and anti-angiogenic factors during pregnancy may have a role in the pathophysiology of pre-eclampsia.* © *2019 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of the International Society of Ultrasound in Obstetrics and Gynecology.*

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INTRODUCTION

Pre-eclampsia is a multisystem disorder defined as new-onset hypertension and proteinuria after the 20th week of pregnancy[1]. Pre-eclampsia occurs in 2–8% of pregnancies and is a major cause of maternal morbidity and adverse perinatal outcome, accounting for 10–15% of maternal deaths[2]. In this life-threatening condition, maternal complications arise from excessive inflammation and endothelial damage to multiple organs, including the placenta[2]. The known risk factors for pre-eclampsia include nulliparity, age over 40 years and pre-existing conditions including hypertension, renal disease and diabetes[2].

Although the exact pathophysiological mechanism is unclear, it is postulated that abnormal placentation in early pregnancy predisposes women to pre-eclampsia[2] and that disturbance in the balance between pro- and anti-angiogenic factors plays an important role[3,4]. Placental growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family and a pro-angiogenic factor, is expressed in the placenta throughout pregnancy[5]. Soluble fms-like tyrosine kinase-1 (sFlt-1) is a potent anti-angiogenic factor that binds VEGFs, including PlGF, thereby inhibiting angiogenesis during pregnancy[6]. An abnormal serum sFlt-1/PlGF ratio, with low concentrations of PlGF and elevated concentrations of sFlt-1, often occurs in pre-eclamptic women and may be a marker of impaired placentation[7,8].

Human chorionic gonadotropin (hCG), a pregnancy-specific hormone produced by trophoblast cells, regulates progesterone production, implantation, uterine growth and immune cell function[9]. hCG also regulates placental development, angiogenesis and vasculogenesis, partially via effects on VEGFs[10–14]. Several studies suggest that high and/or low hCG concentration is a marker of subsequent clinical manifestation of pre-eclampsia[15–18]. However, considerable between-study discrepancies exist, such as the gestational age at hCG measurement, the specific hCG isoform that was assessed and the specific pre-eclampsia phenotype analyzed[15–20]. To our knowledge, no study has investigated the potential effect of early placental function markers that may predispose women to pre-eclampsia, such as the sFlt-1/PlGF ratio, on the association between hCG and the risk of pre-eclampsia.

In the current study, we aimed to investigate the associations of total hCG concentration with the sFlt-1/PlGF ratio and the risk of subsequent pre-eclampsia, in a large population-based prospective cohort.

SUBJECTS AND METHODS

Study population

This study was embedded in Generation R, a population-based prospective cohort study of pregnancies followed from early gestation onwards, in Rotterdam, The Netherlands[21]. This cohort study was designed to assess early environmental and genetic determinants of pre- and postnatal growth, development and health[21]. In this study, 8879 pregnant women with an expected delivery date between April 2002 and January 2006 were eligible and enrolled[21]. Women who had undergone *in-vitro* fertilization ($n = 38$), twin pregnancies ($n = 91$) and women with pre-existing hypertension ($n = 135$) were excluded from the analysis. Total hCG was determined in all first available serum samples and was available in 7754 women[22].

The general design, research aims and specific measurements in the Generation R study have been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam (date: 17 December 2001). Written informed consent was obtained from all participants.

hCG measurements

Total hCG was measured in first available samples (median gestational age, 14.4 weeks; 95% range, 10.1–26.1 weeks) using a solid-phase two-site chemiluminescence immunometric assay, calibrated against World Health Organization Third International Standard 75/537, on an Immulite 2000 XPi system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The assay detects serum intact hCG, hyperglycosylated hCG, serum nicked hCG, serum nicked hyperglycosylated hCG, serum asialo hCG, serum hCG free β-subunit and serum nicked hCG β. The intra-assay variation coefficients were 8.0%, 6.3% and 5.1% at concentrations of 9.7, 53.1 and 821.5 IU/L, respectively. hCG concentration was standardized by calculating SD scores adjusted for gestational age at blood sampling, as described previously[22].

Pre-eclampsia

Pre-eclampsia diagnosis was confirmed by a certified medical doctor, blinded to analyte results, by reviewing hospital charts[23], and was defined, according to the criteria of the International Society for the Study of Hypertension in Pregnancy, as the development of systolic blood pressure (BP) of ≥ 140 mmHg and/or diastolic BP of ≥ 90 mmHg (on at least two BP readings) after 20 weeks of gestation in a previously normotensive woman, and the presence of proteinuria (defined as two or more dipstick readings of ≥ 2, one catheter sample reading of ≥ 1 or a 24-h urine collection containing ≥ 300 mg of protein)[1]. Pregnancy-induced hypertension was defined as development of systolic BP of ≥ 140 mmHg and/or diastolic BP of ≥ 90 mmHg (on at least two BP readings) after 20 weeks of gestation in a previously normotensive woman, without the presence of proteinuria[1].

Angiogenic factor measurements

sFlt-1 and PlGF concentrations were obtained from EDTA blood samples taken in early (< 18 weeks; median, 13.2 weeks; 95% range, 9.6–17.6 weeks) and mid- (18–25 weeks; median, 20.4 weeks; 95% range, 18.5–23.5 weeks) pregnancy and were analyzed using a

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microparticle-enhanced immunoassay on the Architect System (Abbott Diagnostics BV, Hoofddorp, The Netherlands). The between-run coefficients of variation for sFlt-1 were 2.8% at 5.5 ng/mL and 2.3% at 34.0 ng/mL. The between-run coefficients for PlGF were 4.7% at 24 pg/mL and 3.8% at 113 pg/mL. Measurements were available for 70.8% and 87.5% of women for the first and second timepoint, respectively (non-response analysis is shown in Table S1). For measurement, we calculated the ratio of sFlt-1 and PlGF, which is a clinical factor that can be used to predict the risk of pre-eclampsia[7,8].

Covariates

Information on maternal age, educational level, ethnicity and smoking status was obtained by questionnaires during pregnancy. Ethnicity was determined according to the woman's country of origin and defined according to the classification of Statistics Netherlands. Maternal smoking status was classified as non-smoker, smoker until known pregnancy or continued smoker during pregnancy[21]. Information on *in-vitro* fertilization treatment, parity and sex of the child was obtained from community midwifery and hospital registries.

Statistical analysis

The association of hCG with sFlt-1/PlGF ratio was investigated using multiple linear regression models. The association of hCG with pre-eclampsia and pregnancy-induced hypertension was investigated using multiple logistic regression analyses. Restricted cubic splines utilizing three or four knots were used to assess possible non-linearity. Subsequently, to further quantify the risk of pre-eclampsia, odds ratios were calculated for the upper and lower 5th, 10th and 15th percentile cut-offs for hCG concentration. Multivariable associations were depicted graphically by plots, and β-estimates with 95% CI are shown in Table S2. Full model estimates are shown in Tables S3–S5. For linear regression models, the assumption of normality of standardized residuals of the model was tested. Effect modification by gestational age at blood sampling or fetal sex was tested by introducing to the model a product interaction term of hCG × gestational age at blood sampling or hCG × fetal sex, and a *P*-value of < 0.15 was considered for stratification.

All model covariates were selected based on biological plausibility, change in the effect estimate or residual variability of the model. All analyses were adjusted for maternal age, ethnicity, body mass index (BMI), parity, educational level, smoking status and fetal sex.

In order to cope with missing data on the covariates, multiple imputation was performed according to the Markov Chain Monte Carlo method[24]. Before imputation, exploratory analyses were performed by investigating the pattern of missingness for each variable. All variables showed a random missingness pattern. The percentage of missing data was less than 1% for maternal age, BMI, parity and fetal sex, 5.3% for ethnicity, 8.8% for education level and 12.6% for smoking status. Five imputed datasets were created and pooled for analysis. hCG concentration adjusted for gestational age at measurement and the risk of pre-eclampsia were used as predictor variables only. There were no differences in descriptive characteristics between the original and imputed datasets.

Statistical analyses were performed using SPSS Statistics for Windows version 21.0 (IBM Corp., Armonk, NY, USA) and R statistical software version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria; package 'rms').

RESULTS

After exclusions, the final population comprised 7754 women (Figure 1), the characteristics of whom are shown in Table 1. There were 165 (2.2%) women who developed pre-eclampsia, 4287 (55.3%) women who were nulliparous, and the population was predominantly of Dutch origin (3762; 48.5%) (Table 1).

Association of hCG with angiogenic factors

There was a positive association between hCG and sFlt-1/PlGF ratio during early pregnancy ($P < 0.0001$; Figure 2a). The positive association between hCG and sFlt-1/PlGF ratio during mid-pregnancy ($P = 0.0002$, Figure 2b) attenuated after adjustment for sFlt-1/PlGF ratio at inclusion ($P = 0.29$; Figure 2c). There was no association between hCG and Δ (the difference between

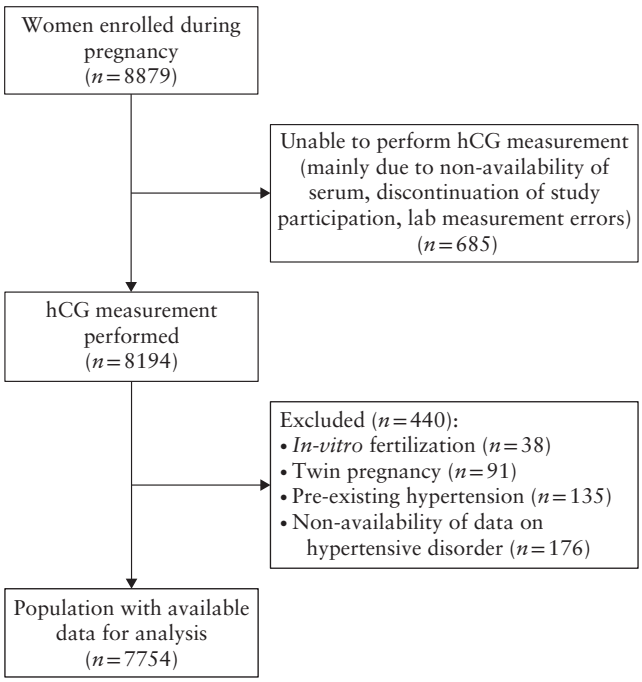

Figure 1 Flowchart showing selection procedure of study population of pregnant women with available data on human chorionic gonadotropin (hCG) and angiogenic factors.

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Table 1 Demographic and clinical characteristics of 7754 pregnancies

Characteristic	*Value*
hCG (IU/L)	35 522.0 (6074.9–99 890.3)
GA at blood sampling (weeks)	14.4 (10.1–26.1)
Hypertensive disorder in pregnancy	
Pre-eclampsia	165 (2.2)
Pregnancy-induced hypertension	286 (3.7)
No disorder	7303 (94.2)
sFlt-1/PlGF ratio	
< 18 weeks	117.0 (19.5–432.6)
18–25 weeks	24.4 (5.3–106.8)
Maternal age (years)	29.6 ± 5.3
BMI (kg/m^2)	24.7 ± 4.4
Parity	
Nulliparous	4287 (55.3)
Primiparous	2325 (30.0)
Multiparous	1142 (14.7)
Smoking status	
Non-smoker	5659 (73.0)
Stopped smoking	676 (8.7)
Smoker	1419 (18.3)
Educational level	
None or primary education	952 (12.3)
Secondary education	3651 (47.1)
Higher education	3151 (40.6)
Ethnicity	
Dutch	3762 (48.5)
Moroccan	557 (7.2)
Turkish	763 (9.8)
Surinamese	705 (9.1)
Asian	441 (5.7)
Other European	598 (7.7)
Other non-European	928 (12.0)
Fetal sex	
Male	3911 (50.4)
Female	3843 (49.6)

Data are shown as median (95% range), *n* (%) or mean ± SD. BMI, body mass index; GA, gestational age; hCG, human chorionic gonadotropin; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

first and second measurements) sFlt-1/PlGF ratio after adjustment for sFlt-1/PlGF ratio at inclusion ($P = 0.29$; Figure S1). The associations of hCG with PlGF and sFlt-1 individually are shown in Figures S2 and S3, respectively.

Association of hCG with pre-eclampsia

Figure 3 shows the association between hCG and the risk of pre-eclampsia. High hCG concentration was associated with a 1.5–2.7-fold increased risk of pre-eclampsia, depending on the hCG cut-off value used (Figure 3). After addition of a product interaction term to the model (hCG × fetal sex, $P = 0.020$), we stratified the association between hCG and the risk of pre-eclampsia according to fetal sex, which showed a stronger association of high hCG with the risk of pre-eclampsia in pregnancies with a male fetus (4.0-fold increased risk in males *vs* 2.2-fold increased risk in females when hCG concentration was greater than the highest 5th percentile; Figure S4). We did not observe effect modification according to gestational age (hCG × gestational age, $P = 0.73$; data not shown). Furthermore, when sFlt-1/PlGF ratio was adjusted for (in the subgroup of women with available data on angiogenic factors), the association between hCG and pre-eclampsia attenuated by 40% (β-estimate change from 0.19 ± 0.10 ($P = 0.052$) to 0.12 ± 0.10 ($P = 0.22$)).

The association of hCG with the risk of pregnancy-induced hypertension (Figure S5) showed a similar trend, although it did not reach statistical significance.

DISCUSSION

In this study, we demonstrated associations of high total hCG concentration with high sFlt-1/PlGF ratio and increased risk of pre-eclampsia. Our results suggest that up to 40% of the association between hCG and pre-eclampsia

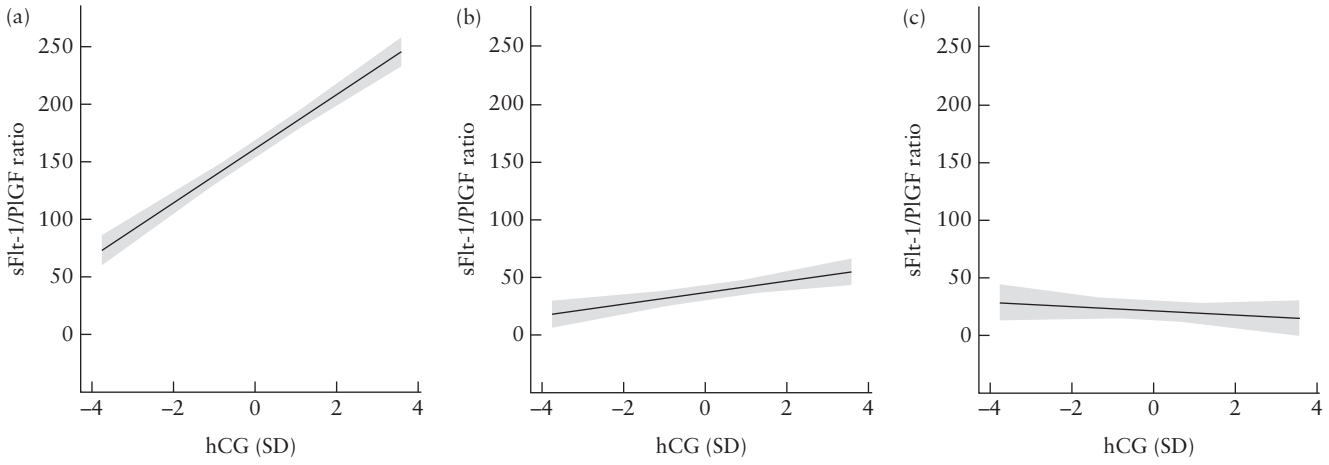

Figure 2 Association of total human chorionic gonadotropin (hCG) concentration with early- (< 18 weeks; $P < 0.0001$ (a)) and mid- (18–25 weeks; $P = 0.0002$ (b)) pregnancy soluble fms-like tyrosine kinase-1/placental growth factor (sFlt-1/PlGF) ratio, and mid-pregnancy sFlt-1/PlGF ratio adjusted for measurement at inclusion ($P = 0.29$ (c)). Plots show predicted mean with 95% CI calculated using linear regression models. Analyses were adjusted for ethnicity, body mass index, maternal age, fetal sex, educational level, parity and smoking status.

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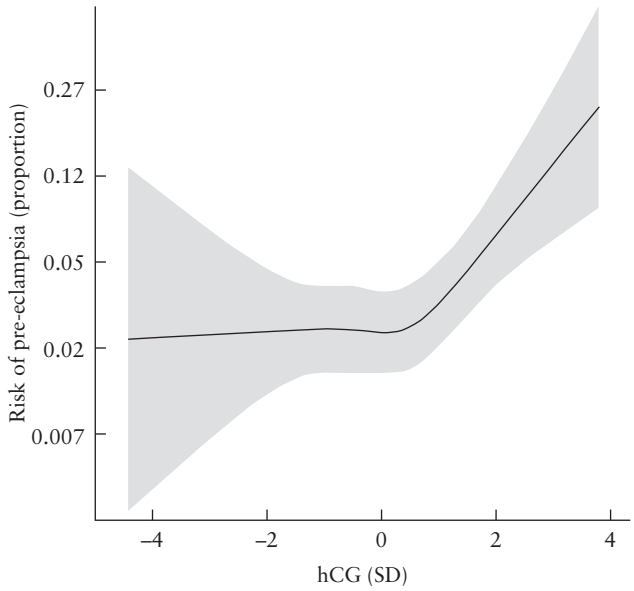

hCG cut-off	OR (95% CI)	*P*
< 5th percentile	0.98 (0.47–2.03)	0.95
< 10th percentile	0.83 (0.47–1.46)	0.51
< 15th percentile	0.89 (0.57–1.41)	0.64
> 85th percentile	1.50 (1.00–2.23)	0.046
> 90th percentile	2.09 (1.37–3.18)	0.001
> 95th percentile	2.73 (1.65–4.50)	< 0.001

Figure 3 Association of total human chorionic gonadotropin (hCG) concentration with risk of pre-eclampsia ($P = 0.0001$). Plot shows predicted mean with 95% CI calculated using regression model. Analyses were adjusted for ethnicity, body mass index, maternal age, fetal sex, educational level, parity and smoking status. Cut-offs for calculation of odds ratios (OR) are based on percentiles of total hCG standardized for gestational age.

might be mediated via effects of hCG on the angiogenic factors sFlt-1 and PlGF.

Pre-eclampsia is an important cause of maternal and fetal morbidity and mortality[1,2,25,26]. Great effort has been put into investigating the pathophysiological mechanisms leading to pre-eclampsia and the clinical markers of this condition[2]. Although the exact mechanism remains to be elucidated, abnormal placentation and a subsequent hyperinflammatory response, characterized by a disturbance in pro- and anti-angiogenic factors, play a major role[2–4]. Multiple studies have investigated the association of hCG with the risk of pre-eclampsia, comprising data on different hCG isoforms[15–20,27]. Although in early and in late pregnancy high total hCG and β-hCG concentrations are suggested as a marker of (subsequent) pre-eclampsia[15–18], some studies report an association of low early-pregnancy β-hCG[19,27] and hyperglycosylated hCG with pre-eclampsia[20]. The interpretation and comparison of these studies are hampered by the differences in hCG measurement. Most studies utilize β-hCG, which is often measured in the setting of screening or as biochemical proof of pregnancy. However, different hCG isoforms predominate during distinct phases of gestation[28,29], and studies measuring only the β-hCG isoform may therefore have different results as compared with those in the current study in which total hCG was measured. Future studies meta-analyzing individual participant data could prove useful in elucidating the magnitude of the effect of these differences on the association with pre-eclampsia and delineate the extent to which discrepancies in associations with clinical outcome could be explained by variation in hCG isoform, gestational age, assay used or, for example, potential placental, endocrine or vascular factors that we could not identify.

In pre-eclamptic women, the sFlt-1/PlGF ratio is higher than in healthy women[7,30,31]. It is considered that high production of sFlt-1 in the placenta largely counteracts the effects of PlGF and thereby contributes to the development of hypertension and proteinuria[6]. In pathological states characterized by high hCG concentration, such as molar pregnancy, which predisposes to pre-eclampsia, the expression of placental sFlt-1 is higher compared with that of PlGF[32,33]. As hCG may play a role in the regulation of placental angiogenesis via effects on the production of VEGFs[10–14], we hypothesized that abnormal hCG concentration may be a contributing factor to the unfavorable balance in the expression of placental pro- and anti-angiogenic factors in women with pre-eclampsia.

There may be an additional underlying mechanism contributing to the association of high hCG concentration with the increased risk of pre-eclampsia, such as hCG-modulating effects on immune cells, which may respond differently depending on the hCG concentration[34]. In general, hCG has a protective effect on pregnancy and promotes fetal survival by promoting immune tolerance via interaction with macrophages, natural killer cells, and B- and T-lymphocytes[34]. However, in higher doses, hCG may exhibit harmful effects on fetal development[35,36] and may induce an immune cell response with altered cytokine and reactive oxygen species production[37], triggering exaggerated sFlt-1 production and thereby reinforcing the cumulative effect of factors predisposing women to pre-eclampsia[38]. Alternatively, higher availability of sFlt-1 and anti-angiogenic effects characterizing pre-eclampsia might be due to hCG-stimulated binding of PlGF to membrane and local circulating receptors[4,10–14]. Moreover, it has been proposed that dysregulation of not only the concentration of

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hCG but also its glycosylation pattern might underlie the pathophysiological events and impaired angiogenic and immune response in pre-eclampsia[39]. Despite its known pro-angiogenic effects in normal pregnancy, the effects of altered hCG concentration on the cross-talk between trophoblast, endothelial cells and the immune system in women with pre-eclampsia are poorly understood[39]. Therefore, future studies should further elucidate the multifactorial risk-factor profile of the association of hCG with pre-eclampsia.

In an attempt to diminish the adverse effects of oxidative stress and hypertension in women with pre-eclampsia, a series of maternal compensatory responses may arise[40,41]. The association of high hCG concentration with an increased risk of pre-eclampsia could therefore also represent a compensatory response of the trophoblast cells to fetoplacental hypoxia, in which high hCG concentration is supposed to stimulate angiogenic factor expression and improve placental function[5,6,10,14]. Another relevant determinant of adaptive mechanisms in pregnancy is fetal sex, as hCG concentration is dependent on fetal sex[42,43]. We demonstrated that in women with high hCG concentration, those with a male fetus have a higher risk of pre-eclampsia than those with a female fetus. This might be explained by a potentially greater susceptibility of male fetuses to the impact of hCG variation, or by fetal-sex-specific differences in the variation of hCG isoforms[22]. The hypothesis of fetal-sex-specific differences is further supported by our previous study that showed that the association of low hCG with lower fetal growth is stronger in pregnancies with a male fetus[44].

We were able to perform analyses using prospectively collected data on measurements of total hCG, rather than only on a single isoform, and also had detailed data available on pre-eclampsia and relevant covariates in a large population. We were limited by the fact that we had only a single hCG measurement available for each woman, rendering us unable to examine inter-individual differences in hCG variation during pregnancy. Future studies may want to assess whether hCG trajectory is associated with pre-eclampsia. Another potential limitation could be the availability of data on PlGF and sFlt-1 in 70.8% of the study population, which could potentially introduce bias. However, a non-response analysis showed no difference in mean hCG concentrations or in the frequency of pre-eclampsia between participants with and those without available data on PlGF and sFlt-1. Finally, the current study is observational and thus does not allow for inference about causality and does not exclude residual confounding.

This study of a healthy population of pregnant women demonstrates that high hCG concentration in early pregnancy is associated with an increased risk of pre-eclampsia. The effect of high hCG concentration on the balance between pro- and anti-angiogenic factors during pregnancy might partially explain this finding. Future studies may want to assess if these findings could be incorporated into clinical prediction models for hypertensive disorders of pregnancy.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Demographic and clinical characteristics in population of 7754 pregnancies, according to availability of sFlt-1 and PlGF data

Table S2 Association of maternal hCG (SD) with sFlt-1/PlGF ratio

Table S3 Linear regression model for association of maternal hCG (SD) with sFlt-1/PlGF ratio at < 18 weeks' gestation

Table S4 Linear regression model for association of maternal hCG (SD) with sFlt-1/PlGF ratio at 18–25 weeks' gestation

Table S5 Linear regression model for association of maternal hCG (SD) with sFlt-1/PlGF ratio at 18–25 weeks' gestation, adjusted for baseline measurement

Figure S1 Association of total hCG concentration with ΔsFlt-1/PlGF ratio between early (< 18 weeks) and mid- (18–25 weeks) pregnancy before and after adjustment for early measurement.

Figure S2 Association of total hCG concentration with early- (< 18 weeks) and mid- (18–25 weeks) pregnancy PlGF concentration.

Figure S3 Association of total hCG concentration with early- (< 18 weeks) and mid- (18–25 weeks) pregnancy sFlt-1 concentration.

Figure S4 Association of total hCG concentration with risk of pre-eclampsia, according to fetal sex.

Figure S5 Association of total hCG concentration with risk of pregnancy-induced hypertension.

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