

Maternal Plasma Hepatocyte Growth Factor Concentrations in Women Who Subsequently Developed Preeclampsia

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Purpose: The aim of this nested case-control study was to investigate the association between hepatocyte growth factor (HGF) concentrations in maternal plasma and the risk of developing preeclampsia.

Materials and Methods: Plasma HGF concentration were measured in 52 women who subsequently developed preeclampsia and 104 normal pregnant women at the time of genetic amniocentesis (15-20 weeks) by enzyme-linked immunosorbent assay.

Results: Maternal plasma HGF concentrations were significantly higher in women with subsequent preeclampsia (median: 737.8 ng/mL vs. 670.4 ng/mL, $P=0.003$) than in normal controls. However, HGF concentrations were not significantly different between subgroups by preeclamptic complications. After adjusting for potential confounding factors, women with HGF concentrations ≥ 702.5 ng/mL had a 3.2-fold increased risk (95% CI 2.7-5.4, $P<0.001$) of subsequent development of preeclampsia compared with women with HGF concentrations <702.5 ng/mL.

Conclusion: Elevated maternal plasma HGF concentrations in the early second-trimester are associated with an increased risk of developing preeclampsia.

Key words: Plasma, Hepatocyte growth factor, Risk factor, Subsequent preeclampsia

Introduction

Preeclampsia, a pregnancy-related syndrome, is characterized by maternal hypertension and proteinuria after 20 weeks of gestation, affecting approximately 5-10% of all pregnancies.¹ This syndrome, a leading cause of maternal and perinatal morbidity and mortality, occurs only in the presence of a placenta

and remits dramatically after the placenta has been delivered.²

Despite extensive research, the pathophysiology of this condition remains unclear. Accumulating evidence, however, indicates that failure of the cytotrophoblast invasion of maternal uterine spiral arteries plays a central role.^{3,4} Consequently, reduction of uteroplacental blood perfusion by shallow implantation results in local placental hypoxia.⁵ Chronic hypoxia

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or alternate periods of hypoxia/re-oxygenation within the intervillous space are expected to trigger tissue oxidative stress and increase placental apoptosis and necrosis.^{6,7)} A hypoxic/ischemic placenta may then release soluble factors, cytokines, and trophoblastic debris into the maternal circulation eventually causing systemic endothelial damage and dysfunction, which leads to the main clinical symptoms of preeclampsia.⁸⁻¹⁰⁾

Hepatocyte growth factor (HGF), a pleiotropic cytokine, regulates cell growth, differentiation, and morphogenesis in many different cell types and tissues. It is also a potent angiogenic factor that stimulates the proliferation and migration of endothelial and smooth muscle cells.^{11,12)} In the human placenta, HGF is expressed in the placental villous core and acts in a paracrine manner on trophoblasts expressing the HGF receptor (HGFR, also known as c-Met).¹³⁾ HGF knockout mutations in mice result in embryonic death in utero due to placental insufficiency.¹⁴⁾ HGF has been postulated to play an essential role in placental development. We hypothesize that plasma HGF concentrations are aberrant in women who subsequently develop preeclampsia prior to clinical manifestation of the symptoms of preeclampsia. In this nested case-control study, we investigated the relationship of the early second-trimester plasma HGF concentrations and the risk of developing preeclampsia.

Materials and Methods

All subjects were recruited prospectively from the Obstetrics and Gynecology Department at Cheil General Hospital in Seoul, Korea between October 2001 and June 2004. Maternal venous blood samples were collected from a cohort of 3,000 consenting women at the time of genetic amniocentesis and diagnosis of preeclampsia. Blood samples and clinical data were collected with the approval of the Ethics Committee at Cheil General Hospital (CGH-IRB-2010-35), and a written informed consent was obtained from all participants. We retrospectively reviewed the medical records of 3,000 recruited pregnant women. A nested case-control study was conducted in 52 women with subsequent preeclampsia and 104 normal pregnant women at the time of genetic amniocentesis (15-20 weeks). At the time of sampling, women with subsequent preeclampsia included in the study were clinically healthy and showed no signs of preeclampsia or other pregnancy complications. Exclusion criteria included prior preeclampsia, spontaneous abortion, intrauterine fetal death, fetal chromosomal or congenital abnormalities, alcohol consumption, smoking, and preexisting medical conditions such

as diabetes, chronic hypertension, autoimmune disease, or renal disease.

Preeclampsia was sub-classified as mild preeclampsia and severe preeclampsia according to disease severity, term preeclampsia (≥ 37 weeks) and preterm preeclampsia (< 37 weeks) according to delivery week, and preeclampsia who delivered an appropriate for gestational age (AGA) neonate and preeclampsia who delivered a small for gestational age (SGA) neonate according to birth weight of a neonate.

Preeclampsia was diagnosed as hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) and proteinuria (≥ 300 mg in a 24 h urine collection and/or $\geq 1+$ on dipstick testing) after 20 weeks of gestation.¹⁵⁾ Severe preeclampsia was diagnosed as diastolic blood pressure ≥ 110 mmHg; severe proteinuria (urinary protein excretion ≥ 5 g per 24 hr and / or $\geq 3+$ on dipstick testing); evidence of pulmonary edema; seizures; oliguria (< 500 mL/day); thrombocytopenia (platelet count $< 100,000$ /mL); and severe central nervous system symptoms such as altered mental status, headaches, blurred vision, or blindness.¹⁵⁾ SGA and AGA were defined by a birth weight below the 10th percentile and a birth weight from the 10th to 90th percentile for gestational age at birth, respectively, according to the national birth weight distribution of the Korean population.¹⁶⁾

A patient was considered to have a normal pregnancy if she met the following criteria: 1) singleton gestation; 2) no medical, obstetrical or surgical complications; 3) absence of labor at the time of venipuncture; and 4) delivery of a normal term infant (≥ 37 weeks) whose birth weight was between the 10th and 90th percentile for gestational age.

Maternal peripheral blood was collected in EDTA-containing tubes and was centrifuged at 2,500g for 10 min within 24 h after sampling. The supernatant plasma was transferred into a 1.5 ml sterile tube, and aliquots of plasma were stored at -80°C until use. Plasma HGF concentrations were measured by ELISA kit (Invitrogen Corporation, Camarillo, CA, USA). The inter- and intra-assay coefficients of variation were 7.2% and 5.4%, respectively.

Statistical analysis was performed using the Statistical Package for Social Sciences version 12.0 (SPSS, Chicago, IL, USA). The clinical data were compared by the Student's t test for continuous variables and by the χ^2 test for categorical variables. A comparison of HGF concentration between the two groups was performed using the Mann-Whitney U test.

Receiver operating characteristic (ROC) analysis was performed to assess the best cut-off value and discriminating capacity of the HGF concentration in the second-trimester plasma for predicting women who subsequently developed preeclampsia. Logistic

regression analysis was used to adjust for the effects of covariates (maternal age, body mass index, nulliparity and gestational age at sampling) and identify independent relationships with results reported as adjusted odds ratios (OR) with 95% confidence intervals (CI). A P value of <0.05 was considered statistically significant.

Results

The clinical characteristics of the study population are shown in Table 1. There were no significant differences in maternal age and gestational age at sampling between women with subsequent preeclampsia and normal controls ($P>0.05$). However, nulliparous women and body mass index (BMI) were higher in women with subsequent preeclampsia compared to the controls ($P<0.05$). At the time of sampling, blood pressures and proteinuria were not significantly different between women with subsequent preeclampsia and controls ($P>0.05$). The gestational age at delivery was earlier and the fetal birth weights were lower in women with subsequent preeclampsia compared to the controls ($P<0.001$).

The maternal plasma HGF concentrations in the second-

Table 1. Clinical Characteristics of the Study Population

	Controls (n=104)	Women with subsequent preeclampsia (n=52)	P
Characteristics at sampling			
Maternal age (y)	33.8±3.9	33.0±3.5	0.163
Nullipara	52 (50.0%)	36 (69.2%)	0.012
BMI (kg/m ²)	21.1±2.5	22.7±3.0	0.002
Gestational age (weeks)	16.8±1.3	17.0±1.3	0.136
SBP (mmHg)	115.3±10.7	118.5±13.0	0.446
DBP (mmHg)	68.6±6.4	70.0±5.6	0.381
Proteinuria (dipstick)	Negative	Negative	-
Characteristics at presentation			
SBP (mmHg)	-	157.7±13.0	$<0.001^a$
DBP (mmHg)	-	100.2±9.5	$<0.001^a$
Proteinuria (dipstick)	-	2.5±0.9	$<0.001^b$
Characteristics at delivery			
Gestational age (weeks)	39.2±1.2	36.0±3.6	<0.001
Birth weight (g)	3378.8±361.1	2456.9±403.8	<0.001

Data are presented as mean±SD or number (percentage).

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

^aBlood pressure at sampling vs. at presentation in women with subsequent preeclampsia

^bProteinuria at sampling vs. at presentation in women with subsequent preeclampsia

trimester (15–20 weeks) were higher in women with subsequent preeclampsia than those in normal controls (737.8 ng/mL (range: 495.8–1508.1 ng/mL) vs. 670.4 ng/mL (range: 387.6–1276.0 ng/mL), $P=0.003$) (Fig. 1). When women with subsequent preeclampsia were sub-classified according to complications, the maternal plasma concentrations of HGF were not significantly different between subgroups (Table 2).

We analyzed the change of HGF concentrations in each group according to gestational age (Fig. 2). The concentrations of HGF was positively correlated with gestational age for control groups ($r=0.256$, $P=0.009$). However, there is no correlation

Table 2. Maternal Plasma HGF Concentrations in Subgroups of Women who Subsequently Developed Preeclampsia

	2 nd trimester HGF concentration (ng/mL)	P
Disease severity		
Mild	732.8 (533.6-1508.1) (n = 15)	0.592
Severe	742.8 (495.8-1279.4) (n = 37)	
Gestational week at delivery		
Term	762.5 (495.8-1508.1) (n = 27)	0.821
Preterm	719.4 (501.0-1318.1) (n = 25)	
Birth weight at delivery		
AGA	749.4 (531.7-1279.4) (n = 32)	0.908
SGA	718.1 (495.8-1508.1) (n = 20)	

Data are presented as median (range).

AGA: appropriate for gestational age; SGA: small for gestational age.

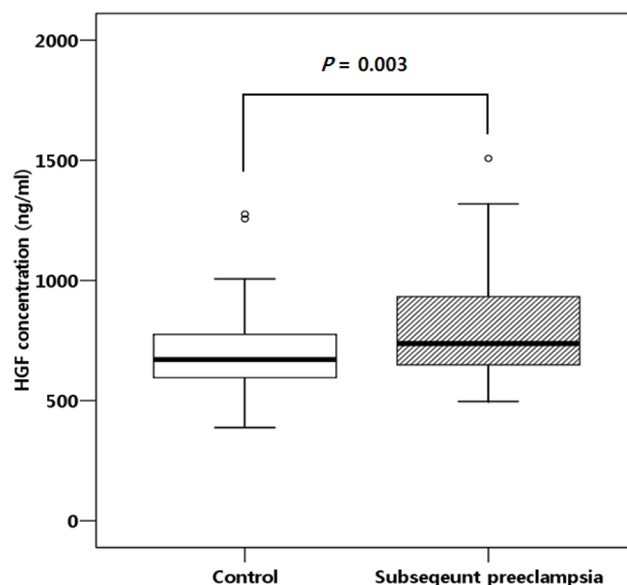


Fig. 1. Box plots of the HGF concentrations in control and subsequent preeclampsia groups in the second-trimester. The upper and lower limits of the boxes represent the 75th and 25th percentiles, respectively. The upper and lower whiskers represent the 90th and 10th percentiles, respectively. The median is indicated by the line in each box. Outliers are indicated by circles.

between gestational age and HGF concentration in women with subsequent preeclampsia ($r=-0.051, P=0.726$).

The maternal plasma HGF concentration with the best cut-off value of 702.5 ng/mL had a specificity of 72% and a sensitivity of 70% (area under the curve [95% CI]: 0.738 [0.643-0.833], $P=0.005$). The logistic regression analysis revealed a significant association between second-trimester HGF concentrations and the risk of developing preeclampsia in the unadjusted and adjusted analyses. In the unadjusted analysis, second-trimester HGF concentration of higher than 702.5 ng/mL was associated with a 2.5-fold increase risk of subsequent development of preeclampsia (95% CI 1.3-4.9, $P=0.009$). After adjusting for maternal age, nulliparity, BMI, gestational age at sampling and gestational age at delivery, HGF in the analysis increased the OR to 3.2 (95% CI 2.7-5.4, $P<0.001$) for women with subsequent preeclampsia.

Discussion

The present study shows that the second-trimester plasma HGF concentrations are higher in women who subsequently developed preeclampsia compared to the normal pregnant women. These elevations are observed at less than 20 weeks of gestation, indicating that elevations in maternal plasma HGF may be useful in identifying women at higher risk for subsequent development of preeclampsia early in gestation.

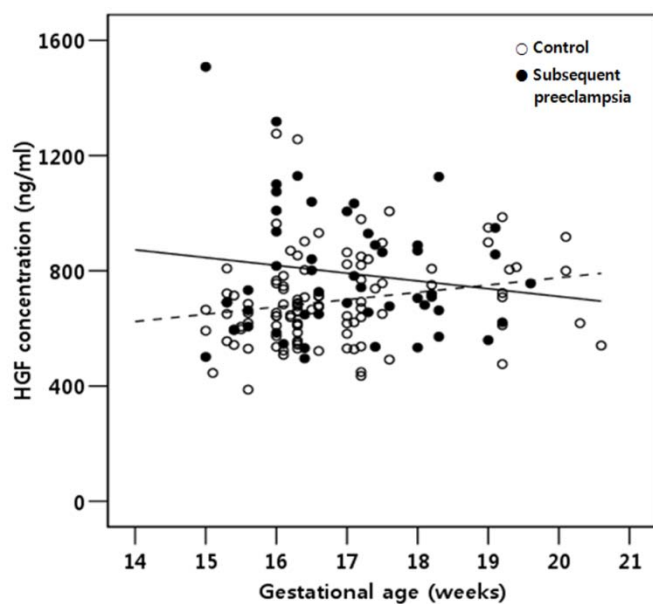


Fig. 2. Correlation between maternal plasma HGF concentration and gestational age in control and subsequent preeclampsia groups. The solid and dashed lines indicate the regression lines for the subsequent preeclampsia and control groups, respectively.

Our results are in line with previous study of Clark et al.,¹⁷ who found that HGF levels start to increase significantly with increasing gestational age from the second half of gestation onwards. They demonstrated that concentrations of circulating HGF in women with intrauterine growth restriction increase gradually during pregnancy and then drop significantly postpartum. In contrast, Tjoa et al. reported that the second-trimester plasma HGF concentrations in preeclamptic patients were not significantly different from normal pregnant women, while those in women with SGA fetuses were significantly elevated.¹⁸ The reasons for this discrepancy are not clear. However, Tjoa et al. used an ELISA kit for HGF from a different manufacturer for use with pregnancy samples.¹⁸ Furthermore, it may be based on the small sample size and differences in the populations studied. Further investigations are needed to confirm these findings and to identify the pathophysiological mechanism underlying the alteration of plasma HGF in preeclampsia.

The HGF/c-Met signaling pathway plays an important role not only in embryogenesis and development but also in angiogenesis.¹⁹⁻²¹ Function of HGF is mediated by the tyrosine kinase receptor, c-Met. Evidences have revealed that the integral c-Met can be released from the endothelial cell membrane by proteolysis to form a soluble, truncated protein (sMet), which is able to bind HGF and disrupt HGF/c-Met signaling.²² At present, it remains unclear whether there exist interactions between HGF and sMet, and we do not know the cause-effect relationship between the circulating sMet and endothelial dysfunction in the occurrence of preeclampsia. Thus, further studies are needed to determine the significance of this substance in the pathophysiology of preeclampsia.

Three facts regarding the placenta in preeclampsia are known: an association with large placentas (excessive trophoblast), a tendency for superficial implantation, and inappropriate trophoblastic immaturity, as assessed by ultrastructural and biochemical criteria.²³⁻²⁵ A unitary hypothesis is that preeclampsia is related to a maturation defect leading to excessive accumulation of inappropriately immature intermediate trophoblast in the placental implantation site. Redline et al. reported that preeclampsia has been associated with an immature hyperproliferative trophoblast in response to reduced uteroplacental flow.²⁶ HGF is known to be a mesenchymal effector for proliferation, differentiation, and function of the uterine epithelial cells.^{27,28} Possibly, the excessive proliferation of cytotrophoblast cells in preeclamptic pregnancies may be accompanied by or preceded by increased HGF production. However, it remains unclear why HGF concentrations were altered in preeclamptic pregnancies. Maternal serum HGF

concentrations have been reported to be increased in women with HELLP syndrome during the third-trimester.²⁹⁾ Although part of the HGF originates from the placenta,³⁰⁾ other organs such as the maternal liver and kidney may equally contribute to the levels of circulating HGF.³¹⁾ It is therefore unlikely that the elevated HGF concentrations found in women with subsequent preeclampsia can be attributed solely to increased placental production. Change of circulating HGF in preeclamptic pregnancies might reflect the much early malfunctions in these maternal organs.

The limitations of this study are that we only included patients from a Korea population. In addition, the number of preeclamptic patients in the second-trimester of pregnancy was relatively small (52 cases, respectively), although it reflects the real prevalence of the early form of the disease, which is $\approx 0.8\%$.³²⁾ Finally, it may be considered that prediction for preeclampsia at 15-20 weeks of gestation is relatively late. Recent studies are focusing in predicting preeclampsia with use of uterine artery Doppler and other biochemical markers in the first-trimester.³³⁻³⁵⁾ Thus, further large-scale, prospective, longitudinal studies are necessary to confirm these results and to assess the usefulness of HGF as an important risk factor for the development of preeclampsia.

In conclusion, we found increased maternal plasma HGF concentrations in women who subsequently developed preeclampsia. Moreover, alterations of the second-trimester HGF concentrations were significantly associated with an increased risk of subsequent development of preeclampsia and could offer insight into disease pathogenesis and the potential for early prediction.

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