Maternal serum IGF-1, IGFBP-1 and 3, and placental growth hormone at 20 weeks’ gestation in pregnancies complicated by preeclampsia

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The authors have nothing to declare.
Abstract

Objective: To investigate whether maternal serum concentrations of placental growth hormone (GH-V), insulin-like growth factor (IGF) 1 and 2, and IGF binding proteins (IGFBP) 1 and 3 were altered in pregnancies complicated by later preeclampsia (PE).

Study design: In a nested case-control study, PE cases (n=71) and matched controls (n=71) were selected from the Screening for Pregnancy Endpoints (SCOPE) biobank in Auckland, New Zealand. Maternal serum hormone concentrations at 20 weeks of gestation were determined by ELISA.

Results: We found that maternal serum GH-V concentration at 20 weeks of gestation was unaltered in the PE group, compared to the control group (median, 1.78 ng/ml vs. 1.65 ng/ml, p = 0.884). Maternal IGF-1 and IGFBP-3 concentrations and the IGF-1/IGFBP-3 ratio in PE pregnancies were significantly higher than in controls (median, 253.1 ng/ml vs. 204.3 ng/ml, p < 0.0001; 8535 ng/ml vs. 7711 ng/ml, p = 0.0023; 0.032 vs. 0.026, p < 0.0001, respectively), whereas maternal IGFBP-1 concentration was significantly lower in PE pregnancies than in controls (median, 34.85 ng/ml vs. 48.92 ng/ml, p = 0.0006).

Conclusion: Our findings suggest a potential role of IGFs and IGFBPs in the prediction of pregnancies complicated by PE. However, the maternal serum concentration of GH-V at 20 weeks’ gestation is unlikely to be useful in the early prediction of PE.
Introduction

Preeclampsia (PE) is one of the leading causes of maternal, fetal, and neonatal mortality and morbidity. It affects 3–5% of pregnancies worldwide and is characterized by maternal hypertension, proteinuria, and if left untreated it can progress to maternal multi-organ failure, coagulopathy and seizures [1]. Women whose pregnancies are complicated by PE also have an increased risk of diabetes mellitus, chronic hypertension, ischemic heart disease, cerebrovascular disease and kidney disease later in life [2, 3]. However, there are no therapeutic approaches available for treatment currently and there are limited options for prevention of PE in women identified to be at high risk [4]. Therefore, identification of biomarkers that predict the development of PE or improve detection of this life-threatening pregnancy disorder is warranted in order to enable better monitoring of patients and reduce the occurrence of adverse complications for them and their babies.

The human growth hormone (GH) locus contains two evolutionarily related growth hormone genes: the pituitary GH gene (GH-N; GH1) and a gene for the placental growth hormone variant (GH-V; GH2) [5, 6]. GH-V binds the GH receptor with similar affinity to GH-N, but has lower affinity for the prolactin receptor [5, 6]. During pregnancy, GH-V is secreted from the syncytiotrophoblast cells of the placenta, and gradually replaces GH-N as the main form of GH in the maternal circulation [5, 6]. Despite this intriguing switch in expression from pituitary to placental expression of GH, very little is known about the role GH-V plays in human pregnancy. However, it is thought that GH-V plays a role in maternal adaptation to pregnancy. Studies investigating the association between maternal serum GH-V and PE are limited, and results have been conflicting. Two studies observed increased GH-V levels in maternal serum at mid to late gestation in pregnancies complicated by PE [7, 8]. Sifakis et al. found no changes in GH-V concentration in maternal serum taken at 11-13 weeks in PE cases.
when compared to controls [9]. Mannik *et al.* demonstrated reduced GH-V expression in placentas from pregnancies complicated by PE [10].

In the current study, we aimed to determine whether there was an association between maternal serum concentration of GH-V at 20 weeks of gestation and the subsequent development of PE. We hypothesised that maternal serum GH-V concentrations were altered in pregnancies with later PE. Serum concentrations of related GH/insulin-like growth factor (IGF) axis proteins: IGF-1, IGF-2, and their binding proteins (IGFBP) 1 and 3 were also measured.

**Materials and Methods**

Ethical approval was obtained from New Zealand Health and Disability Ethics Committees (AKX/02/00/364/AM03), and all women provided written informed consent. Between November 2004 and October 2007, 2,032 nulliparous women with singleton pregnancies were recruited to the Screening for Pregnancy Endpoints (SCOPE) study in Auckland, New Zealand. The inclusion criteria has been described previously [11]. Participants were interviewed and examined by a SCOPE research midwife at 15 and 20 weeks of gestation. At the first visit, detailed clinical and demographic data were collected and entered into an internet accessed, central database with a complete audit trail (MedSciNet, Stockholm, Sweden). A family history of gestational hypertensive disorders was defined as a mother and/or sister(s) who had a history of either gestational hypertension or PE. Mean arterial pressure (MAP) was measured by mercury or aneroid sphygmomanometer at the 15 and 20 weeks SCOPE research visits. Umbilical artery resistance index (RI, defined as maximum – minimum velocity/maximum velocity) and mean uterine artery RI were measured using Doppler ultrasound at 20 weeks. Maternal serum samples were collected at 20 weeks and stored at -80 °C. The specimens did not undergo any freeze/thaw cycles prior
to these analyses. Birth weight was recorded to the nearest gram using electronic scales at the
time of birth.

In this nested case-control study, 71 PE cases were selected from the New Zealand SCOPE
cohort and matched by ethnicity, age (± 3 years) and body mass index (BMI) (± 3 kg/m²) to
71 controls with uncomplicated pregnancies. PE was defined as gestational hypertension
(systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on at least 2
occasions 4 hours apart after the 20 week visit in previous normotensive women) with either
proteinuria (≥ 2+ dipstick or urine protein creatinine ratio ≥ 30 mg/mmol or 24 hours urinary
protein excretion ≥ 0.3 g) or multisystem disease (thrombocytopenia, renal insufficiency,
impaired liver function, pulmonary, cerebral or visual symptoms) [12].

Materials
Recombinant human GH-V (22 kDa) was purchased from Protein Laboratories Rehovot
(Rehovot, Israel) and was reconstituted in 0.4% NaHCO₃ pH 9 [13]. Human GH-V
monoclonal antibodies 78.8E8 (E8; MCA5827G) and 78.7C12 (7C12; MCA5828G) were
obtained from Bio-Rad AbD Serotec (NC, US). E8 does not across react with GH-N or
prolactin; 7C12 shows some cross reactivity with GH-N (5%) as per manufacturer’s
documentation. Antibody 7C12 was biotinylated using a LYNX Rapid Biotin Antibody
Conjugation Kit (Bio-Rad AbD Serotec) according to the manufacturer’s instructions.

GH-V ELISA procedure
We have previously described the development and validation of an in-house enzyme-linked
immunosorbent assay (ELISA) for the measurement of GH-V in serum [14]. In brief,
microtiter plates were coated with antibody E8 diluted in phosphate buffer (0.1M Sodium
Carbonate, pH 9.5) at a concentration of 2 µg/ml by overnight incubation at 4°C. Coated
plates were washed three times with wash buffer (PBS-T; 10 mM phosphate buffer pH 7.4,
150 mM NaCl, 0.05% Tween 20). Blocking was achieved by 1 hour incubation at room
temperature with Ultrablock (Bio-Rad AbD Serotec). Standards were prepared from GH-V solution with a range from 5 to 0.078 ng/ml. Standards and 1:2 diluted serum samples were incubated for 2 hours at room temperature, then washed three times. 8 µg/ml biotinylated antibody C12 was added and incubated for 1 hour. After being washed three times, 200 ng/ml horseradish peroxidase conjugated streptavidin (Bio-Rad AbD Serotec) was added and incubated for 30 min. The microtiter plates were washed four times. End-point detection was processed by using 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) Substrate Reagent Set (BD Biosciences) and stop solution (2N H₂SO₄). Absorbance was read at 450nm and 590 nm within 30 min of stopping reaction. Serum samples were spiked with GH-V and the average recovery rate was 106%. Coefficients of variation (CV) of intra-assay and inter-assay were 4.8% and 6.8%, respectively.

Serum analysis

Serum total IGF-1, total IGF-2, IGFBP-1 and IGFBP-3 were assayed with human-specific ELISA as per the manufacturer’s instructions (Mediagnost, Germany).

Statistical analysis

GH-V, IGF-1, IGFBP-1 and IGFBP-3 data were log-transformed to improve the approximation of normal distribution and linearize relationships. Data are expressed as means ± standard deviation (SD) and median unless stated otherwise. Group means were compared using a Student’s t test. Categorical variables were compared using chi-square or Fisher’s exact test. Pearson’s coefficient was used to determine correlations between variables, presented as r values. All analyses were conducted using IBM SPSS Statistics 21. A p-value of <0.05 was accepted as statistically significant.

Results
The demographic and clinical details are shown in Table 1. There were no significant differences between the two groups except for MAP at 15 and 20 weeks’, birth weight and gestational age at birth.

**Serum concentrations of maternal GH-related hormones**

Maternal serum GH-V concentrations varied considerably between individuals (range: 0.69-8.37 ng/ml). There was no significant difference in maternal serum GH-V concentrations at 20 weeks of gestation in the PE group when compared to the control group (median, 1.78 ng/ml vs. 1.65 ng/ml, p = 0.884) (Fig. 1A). Maternal IGF-1 and IGFBP-3 concentrations and the IGF-1/IGFBP-3 ratios in PE pregnancies were significantly higher than in control (median, 253.1 ng/ml vs. 204.3 ng/ml, p < 0.0001; 8535 ng/ml vs. 7711 ng/ml, p = 0.0023; 0.032 vs. 0.026, p < 0.0001, respectively) (Fig. 1B, E and F). Maternal IGFBP-1 concentrations were significantly lower in PE pregnancies compared to the controls (median, 34.85 ng/ml vs. 48.92 ng/ml, p = 0.0006) (Fig. 1D). There was no significant difference in IGF-2 concentrations between groups (Fig. 1C).

The PE group was separated into early-onset PE patients (defined as PE that developed before 34 weeks of gestation) and late-onset PE (defined as PE which developed at or after 34 weeks of gestation). Early-onset PE patients had increased IGF-1 and IGFBP-1 concentrations compared to late-onset PE patients (IGF-1: median, 313.5 ng/ml vs. 251.3 ng/ml, p =0.0268; IGFBP-1: median, 9372 ng/ml vs. 8461 ng/ml, p = 0.0469), although the number of patients in the early-onset PE group was much lower (n= 8 vs. n= 63).

**Correlation analysis**

In both the control and PE groups, maternal IGF-1 concentrations were positively related to the changes in IGFBP-3 but negatively related to IGFBP-1 concentrations. There was also an association between the concentrations of GH-V and IGF-1 in the control group, but not in the PE group (Table 2).
Interestingly, in the PE group, maternal IGF-1 had a weak positive association with MAP at 15 and 20 weeks’ (r = 0.276, p = 0.021; r = 0.24, p = 0.046); while maternal GH-V was negatively associated with mean uterine artery RI (r = -0.367, p = 0.002) but not with umbilical artery RI (r = 0.043, p = 0.72) at 20 weeks. The associations of IGF-1 with MAP and of GH-V with uterine artery RI were still significant after adjusting for maternal age, ethnicity, BMI, family history of gestational hypertensive disorders, smoking and drinking habits.

**Discussion**

The precise aetiology of PE remains elusive. A long standing hypothesis is that PE develops as a consequence of an immunologically-initiated impaired trophoblast invasion, shallow implantation and inadequate remodelling of the uterine spiral arteries, leading to a high-resistance uteroplacental circulation [15, 16]. Subsequent oxidative stress and inflammation in the placenta alters expression of pro-inflammatory, anti-angiogenic and angiogenic factors, contributing to endothelial cell dysfunction and an inflammatory response [17]. Trophoblast migration and invasive capacity has been shown to be modulated by a number of factors, including oxygen concentration [18], interleukin and transforming growth factor [19], IGF-2 and IGFBP-1 [20], epidermal and hepatocyte growth factors [21, 22]. A potential role of GH-V in the regulation of trophoblast invasion is also suggested by the presence of the GH receptor in the placenta [23] and the stimulation of trophoblast invasion by GH-V in vitro [24]. Further, GH-V is a target gene of peroxisome proliferator-activated receptor-γ (PPARγ) and has been proposed to be involved in the PPARγ-mediated inhibition of trophoblast invasion in an autocrine manner [25]. It is possible that circulating GH-V concentrations may not reflect the effect of GH-V on trophoblast invasion.
Doppler ultrasonography can assess uteroplacental and fetoplacental blood flows and has been suggested as a screening method for PE as increased uterine artery blood flow resistance had been observed prior to the onset of PE [26-28]. One study by Schiessl et al. demonstrated a strong correlation between decreasing uterine and peripheral arterial resistance and increasing maternal serum GH-V concentration [29]. In our study, we found that maternal GH-V concentration was negatively associated with mean uterine artery RI in preeclamptic pregnancies, suggesting a potential role of GH-V in the alteration of maternal arterial resistance.

IGF-1 and IGF-2 mediate a range of actions in many tissues including stimulation of cell growth, cell survival and differentiation. During human pregnancy, maternal IGF-1 is believed to originate mainly from the maternal liver, and GH-V has been suggested to be a main regulator of its synthesis as serum concentrations during pregnancy are highly correlated [30-32]. The majority of circulating IGF-1 is bound to IGFBP-3 and the acid labile subunit to form a 150 kDa ternary complex and is the major storage form of IGF-1 in the circulation [33]. This complex prolongs the half-life of circulating IGF-1 and facilitates its endocrine actions. IGF-1/IGFBP-3 ratio correlates with the amount of free, biologically active IGF-1[34]. IGFBP-1 binds to only a small proportion of circulating IGF-I but is considered to be important for short-term regulation of IGF bioactivity [35, 36]. The placenta expresses considerable amounts of IGFs and IGFBPs; however, it is unclear if the placenta-derived IGFs and IGFBPs serve local function by paracrine or autocrine regulation, or if they are secreted into the maternal or fetal circulation. Nevertheless, IGFs and IGFBPs are crucial for fetal growth and placental development since they regulate trophoblast migration at the maternal-fetal interface [20, 37]. In addition, previous studies also provide evidence in support of a potential role of IGFs and IGFBPs in pregnancies complicated by PE. Increased IGF-2 and decreased IGFBP-1 mRNA expression were observed in the placentae of women
with PE [38, 39]. Transgenic mice overexpressing human IGFBP-1 exhibit a PE phenotype [40]. However, maternal serum concentrations of IGFs and IGFBPs in PE pregnancies vary across different studies. Maternal serum IGF-1 and IGFBP-1 were decreased and IGFBP-3 was increased at 11-13 weeks in pregnancies that subsequently developed PE in several studies [41-43]. However, two studies observed decreased IGF-1 and IGFBP-3, as well as increased IGFBP-1 levels in the third trimester in PE patients [44, 45]. Consistent with our study, increased IGF-1 and decreased IGFBP-1 from the first to second trimester were associated with a higher risk of PE [46, 47]. Further, a progressive increase in maternal circulating IGFBP-1 concentrations from 16 to 36 weeks’ was also observed in those pregnancies complicated by PE [48]. Contributing factors to this observed variation may include the sample size, the time of sampling and the onset and severity of PE where placental integrity is compromised, and consequently the secretion of placental hormones is also compromised.

There is clear evidence that maternal MAP in the second trimester is associated with the later development of PE, although the predictive ability of blood pressure alone is low [49-51]. The vasoactive effects of IGF-1 indicate that IGF-1 can influence blood pressure [52, 53]. Increased IGF-1 levels have also been reported in patients with hypertension [54, 55]. In our study, we found that maternal IGF-1 concentration was positively associated with MAP in the PE group only. However, the role of circulating IGF-1 in the pathogenesis of PE still needs further investigation.

Our study provides further evidence for differences in concentrations of IGF-1 and IGFBPs at 20 weeks of gestation in pregnancies with later PE. However, maternal GH-V concentration was not altered and is unlikely to be useful in the early prediction of PE.

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Conflict of Interest

The authors declare that they have no conflict of interest.
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Figure Legends

Fig. 1. Serum GH-V, IGF-1, IGF-2, IGFBP-1, IGFBP-3 concentrations and IGF-1/IGFBP-3 ratio.
Data are shown as Tukey box-whisker plots (median, 25th centile, 75th centile and range). Outliers are presented as hollow symbols. *p < 0.05.