Insulin-like Growth Factors-I and -II and Insulin-like Growth Factor Binding Protein-1 during normal pregnancy: pattern of secretion and correlation with other placental hormones

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(Index words: Oestradiol, progesterone, prolactin, placental lactogen, chorionic gonadotrophin)

Abstract

Objectives To describe pattern of secretion of insulin-like growth factor (IGF)-I, IGF-II, IGF binding protein (IGFBP)-1 and their correlation with each other and major placental hormones during normal pregnancy.

Design Longitudinal study.

Setting Academic Institutions and a Tertiary Care Maternity Hospital.

Participants Healthy women with singleton uncomplicated pregnancies (N=35).

Measurements Serum levels of IGF-I, IGF-II, IGFBP-1, chorionic gonadotrophin (HCG), placental lactogen (HPL), prolactin, oestradiol and progesterone were studied thrice during the antenatal period and within 24 h of delivery.

Results IGF-I, IGFBP-1, HPL, prolactin, oestradiol and progesterone increased and HCG decreased significantly with advancing gestation (Repeated measures ANOVA: $P < 0.01$ to 0.0001). IGF-II levels were not significantly affected by period of gestation. Significant negative correlations (multiple regression analysis) were seen between IGFBP-1 and prolactin at 28 ± 2 ($P = 0.0226$) and 36 ± 2 ($P = 0.0417$) weeks of amenorrhoea (WOA) and between oestradiol and IGF-II at 36 ± 2 WOA ($P = 0.037$). Prolactin and IGF-I at 14 ± 2 WOA ($P = 0.0225$) and progesterone and IGFBP-1 at 28 ± 2 WOA ($P = 0.0216$) correlated positively.

Conclusions Maternal IGF-I and IGFBP-1 but not IGF-II significantly increase as pregnancy advances. Components of the IGF system regulate or are affected by some of the placental hormones and the effects vary with the period of gestation.

Introduction

Insulin-like growth factors (IGF)-I and -II are single chain polypeptides with considerable structural homology to each other and to insulin. IGFs circulate in blood bound

to one of several binding proteins known to affect their bioavailability. Pregnancy associated changes in IGF-I and -II and IGFBP-1 and the role of these in fetal growth have been studied by many investigators [1-4]. These studies demonstrated a progressive increase in maternal IGF-I levels as pregnancy advances, but evidence for a similar rise in IGF-II and IGFBP-1 remains inconsistent [5, 6]. Studies that report a pregnancy associated rise in IGFBP-1 describe different patterns of secretion [7-10]. Furthermore IGFBP-1 is known to exist as phosphovariants with the highly phosphorylated form exerting a greater inhibitory effect on IGF-I action [7, 11].

The relationship of IGFs and their binding proteins to other placental hormones has not been adequately investigated in women. In baboons, IGF-I but not IGF-II production is regulated by oestrogen during pregnancy [12, 13]. The only study that reported a non-linear relationship between serum oestrogen and IGF-I in pregnant women was mainly a cross-sectional study with only four subjects from whom serial blood samples were collected [14]. In vitro, IGF-I regulates secretion of prolactin, placental lactogen and IGFBP-1. IGF-I stimulated prolactin secretion by cultured endometrial stromal cells and decidual cells, and placental lactogen secretion by placental explants, and inhibited IGFBP-1 secretion from decidual cells [15-18]. Furthermore, progesterone stimulated IGFBP-1 secretion from the endometrium [19]. The present investigation was carried out to describe patterns of secretion of IGF-I, IGF-II and IGFBP-1 during uncomplicated singleton pregnancies in healthy women and to examine the in vivo correlation of IGF-I, IGF-II and IGFBP-1 to each other and to major placental hormones.

Materials and methods

Thirty-five women aged 21 to 35 years and in their first or second naturally conceived pregnancy were studied. They did not have any chronic or acute illness. Pregnancies were singleton and uncomplicated. The study was approved by the Institutional Review Board and

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informed consent was obtained from study participants. Study protocol has been described in detail previously [20]. Each woman was seen at 14 ± 2 , 28 ± 2 and 36 ± 2 weeks of amenorrhoea (WOA) and within 24 h of delivery. Height, weight, systemic arterial blood pressure and symphysio-fundal height were recorded (if appropriate) at each time and a 5 ml venous blood sample was obtained. Serum was separated and stored in aliquots at -20°C until assayed for IGF-I, IGF-II, IGFBP-1, placental lactogen (HPL), chorionic gonadotrophin (HCG), prolactin (PRL), oestradiol (E2) and progesterone. Samples were analysed in duplicate and all the samples from one subject were analysed within the same assay.

IGF-I, IGF-II, IGFBP-1 and other hormones were measured using following commercially available enzyme immunoassay (ELA) reagents. IGF-I EIA 2947, HCG EIA 1911, HPL EIA 1283 and progesterone EIA 1561 (DRG International, Mountainside, NJ, USA); IGF-II ELISA 10-2600 (Diagnostic Systems Laboratories, Inc., Webster, Texas, USA); IGFBP-1 IEMA 10851-ETMB (Oy Medix Biochemica Ab, Asematie, Kaunianien, Finland); prolactin EIA and direct oestradiol EIA (Immunometrics UK Ltd, Munster Road, Fulham, London). IGF-I assay used measured total IGF-I. IGFBP-1 assay measured predominantly nonphosphorylated and less phosphorylated forms. HCG assay was β -HCG specific. All assays were performed

according to the manufacturer's recommendations. Intra-assay and inter-assay coefficients of variation varied from 3.50% to 8.72% and from 4.30% to 9.14% respectively.

Statistical analyses were performed using Prism 2.01 software (GraphPad Prism, San Diego, California, USA). Effect of WOA on serum concentrations of analytes was tested using repeated measures ANOVA on log transformed antenatal data with post-test for linear trend. Spearman rank correlation was used to identify univariate correlations. When a correlation between two analytes resulted in a P value ≤ 0.5 , these were tested in multiple regression analysis to examine correlations between two analytes while accounting for the effect of other analytes.

Results

Thirty women were in their first pregnancy and the remaining 5 in their second pregnancy. All were Sinhalese in ethnicity. On admission to the study at 14 ± 2 WOA they were (mean $\pm$ SEM) 25.52 ± 0.64 years of age, 46.31 ± 1.13 kg in weight with a body mass index of 19.60 ± 0.42 kg/m² and a symphysio-fundal height of 14.25 ± 0.27 cm. Among the babies born, 17 were males. Newborn anthropometric variables were above the 10th percentile and are described elsewhere [20].

Figure 1. Maternal serum levels (geometric means and 95% confidence limits) of IGF-I, IGF-II (A), IGFBP-1, prolactin (B), oestradiol, progesterone (C), HCG and HPL (D) during normal uncomplicated singleton term pregnancy in healthy mothers (N=35). De: within 24 h of delivery.

Table 1. Results of repeated measures ANOVA with post test for linear trend for different analytes during the antenatal period

Analyte	Repeated measures ANOVA		Post test for linear trend	
	F value	R ²	Slope	R ²
IGF-I	146.1	0.820***	0.163	0.471**
IGF-II	2.890	0.079	-	-
IGFBP-1	7.265	0.185*	0.068	0.061**
Oestradiol	151.7	0.817***	0.300	0.598***
Progesterone	177.6	0.843***	0.315	0.652***
HPL	263.8	0.889***	0.473	0.706***
PRL	125.6	0.792***	0.295	0.502***
HCG	69.02	0.670***	-0.360	0.334***

* P < 0.01, ** P < 0.001, *** P < 0.0001

Table 2. Significant correlations observed between different analytes during multiple regression analysis (WOA-weeks of amenorrhoea)

Dependent variable	WOA	Independent variables used in the model	Independent variable(s) giving significant correlations		
			Variable	β coefficient	P value
IGF-I	14 $\pm$ 2	IGF-II, Oestradiol, HPL, PRL, HCG	PRL	0.012	0.023
IGF-II	36 $\pm$ 2	IGFBP-1, Oestradiol, Progesterone	Oestradiol	-18.41	0.027
IGFBP-1	28 $\pm$ 2	IGF-II, Oestradiol, Progesterone, PRL	Progesterone	0.035	0.022
			PRL	-0.009	0.025
Oestradiol	36 $\pm$ 2	IGF-II, Progesterone, HPL	IGF-II	-0.0067	0.049
Progesterone	28 $\pm$ 2	IGFBP-1, Oestradiol, HPL, PRL	IGFBP-1	0.5237	0.016
Progesterone	36 $\pm$ 2	IGF-I & -II, HCG, Oestradiol, HPL, PRL	HPL	13.33	0.042
HPL	36 $\pm$ 2	IGF-I, Oestradiol, PRL, Progesterone, HCG,	Progesterone	0.01	0.048
			HCG	0.0001	0.0001
PRL	28 $\pm$ 2	IGF-II, IGFBP1, Progesterone	IGFBP-1	-18.93	0.023
PRL	36 $\pm$ 2	IGF-I, IGFBP-1, Progesterone, HPL	IGFBP-1	-15.72	0.042
HCG	36 $\pm$ 2	Progesterone, HPL	HPL	3891	0.0001

Maternal serum IGF-I, IGF-II, IGFBP-1, prolactin, oestradiol, progesterone, HCG and HPL levels (geometric mean and 95% confidence intervals) are shown in Figure 1. Results of the repeated measures ANOVA with post test for linear trend are summarised in Table 1. As expected all analytes tested except HCG increased with advancing pregnancy. HCG significantly decreased and 67% of the variation in antenatal levels was attributed to WOA. Of the analytes that increased with advancing pregnancy, increase with WOA was statistically significant for all analytes except IGF-II. WOA contributed to 82%, 7.85% and 18.5% variation in IGF-I, IGF-II and IGFBP-1 respectively, and to nearly 80% or more of the variation in oestradiol, progesterone, prolactin and HPL.

Univariate correlation tests showed significant negative correlations between IGF-II and oestradiol at 36 ± 2 WOA ($r = -0.3648$, $P = 0.0156$), and between IGFBP-1 and prolactin at 28 ± 2 ($r = -0.417$, $P = 0.0141$) and 36 ± 2 ($r = -0.3698$, $P = 0.0313$) WOA. Significant positive correlations were seen between IGFBP-1 and progesterone at 28 ± 2 WOA ($r = 0.3567$, $P = 0.0416$), IGF-I and prolactin at 14 ± 2 WOA ($r = 0.3737$, $P = 0.0321$), oestradiol and progesterone at 14 ± 2 WOA ($r = 0.399$, $P = 0.0194$), and progesterone and HPL at 36 ± 2 WOA ($r = 0.3503$, $P = 0.0211$).

Results of multiple regression analysis are summarized in Table 2. When accounted for the other analytes IGFBP-1 showed a significant negative relationship with prolactin at 28 ± 2 and 36 ± 2 WOA. However when IGFBP-1 was considered as the dependent variable the negative relationship with prolactin was limited to 28 ± 2 WOA. Similarly oestradiol showed a significant negative relationship with IGF-II at 36 ± 2 WOA which persisted even when oestradiol was considered the dependent variable. IGFBP-1 and progesterone showed a significant positive correlation at 28 ± 2 WOA irrespective of which analyte was the dependent variable. Prolactin correlated positively with IGF-I at 14 ± 2 WOA when IGF-I was the dependent variable, but this relationship failed to hold when prolactin was the dependent variable. A positive relationship between progesterone and HPL persisted at 36 ± 2 WOA irrespective of which analyte was the dependent variable. Positive correlation seen between oestradiol and progesterone in univariate correlation became non significant in the multiple regression analysis. Furthermore when accounted for other analytes HPL and HCG showed a significant correlation at 36 ± 2 WOA irrespective of which was considered the dependent variable.

Discussion

Our study showed a significant increase in maternal serum levels of IGF-I and IGFBP-1 but not IGF-II as pregnancy advanced. Absolute values of these have been reported elsewhere [20]. All other hormones studied followed the pattern of secretion expected during pregnancy.

Several investigators have reported a progressive rise in IGF-I as pregnancy advances [6, 10, 21]. We have further confirmed this and showed that week of amenorrhoea accounts for 82% of the variation in antenatal IGF-I levels. Reports on a pregnancy related rise in IGF-II are inconsistent [6, 22]. Similar to some previous investigators [6], we did not find a significant increase in maternal IGF-II levels as pregnancy advanced. Week of amenorrhoea contributed to less than 10% variation in IGF-II levels.

The progressive rise in IGFBP-1 secretion we observed is similar to the pattern reported by some [7], but not other studies [8-10]. Peak levels of IGFBP-1 between 12 to 13 weeks, a rise from early to mid gestation with declining levels between 24 to 34 weeks and a rise from 2nd to 3rd trimester are patterns of IGFBP-1 secretion reported by others [8-10]. Differences in experimental methodology or assay methods used may account for these discrepancies. IGFBP-1 assay we used was highly specific for nonphosphorylated and less phosphorylated variants. A progressive rise in all three phosphoforms through the three trimesters has been reported in a cross-sectional study [7]. Others have not reported the specificity of IGFBP-1 assays for different phosphoforms [8-10]. In our study antenatal levels of IGFBP-1 were less affected by week of amenorrhoea than IGF-I or other placental hormones. This too could account for inconsistent observations previously reported.

Increase in serum concentrations of prolactin, HPL, progesterone and oestradiol and decrease in HCG as pregnancy advances are well known. We quantified the variation of antenatal levels contributed by week of amenorrhoea and this was nearly 80% or more for prolactin, HPL, oestradiol and progesterone and 67% for HCG.

IGF-I increases prolactin and HPL, and decreases IGFBP-1 secretion in vitro [15-18]. We observed a significant positive correlation of IGF-I only on prolactin secretion in vivo. However, in multiple regression analysis this correlation did not hold when prolactin was the dependent variable but persisted only when IGF-I was the dependent variable. A biological basis for this is not clear.

As pregnancy advanced a negative effect of IGFBP-1 on prolactin secretion appeared to dominate. Increased IGFBP-1 is likely to reduce bioavailability of IGF-I and hence the expected rise in prolactin in response to IGF-I. However IGF-I per se did not appear to have an effect on prolactin secretion. Phosphoforms of IGFBP-1 that we measured are predominantly the forms reported to facilitate IGF-I action. Highly phosphorylated variant with inhibitory effects on IGF-I also may have increased in parallel with these variants. Direct effects of IGFBP-1 independent of IGF-I have been previously reported [23]. Hence a direct inhibition of prolactin secretion by nonphosphorylated and less phosphorylated forms cannot be excluded. The positive correlation between

IGFBP-1 and progesterone at mid gestation agrees with previous reports on in vitro stimulatory effects by progesterone [19]. Reasons for absence of a similar relation during early or mid gestation are not clear.

An inhibitory effect of oestradiol on IGF-I but not on IGF-II was observed in pregnant baboons [12, 13]. A non-linear relationship between oestrogen and IGF-I was observed in pregnant women [14]. We observed a negative correlation between oestradiol and IGF-II but not IGF-I during late pregnancy. IGF-II levels did not significantly differ between early, mid and late pregnancy, despite the significantly increasing oestradiol levels, suggesting that high levels of oestradiol in late pregnancy inhibit IGF-II secretion.

Positive correlation between progesterone and HPL in late gestation agrees with previously reported stimulatory effect of progesterone [25]. Why this correlation is absent at other times is not clear. Persistence of the positive correlation in late gestation even when progesterone was considered the dependent variable, and the positive correlation that persisted between HCG and HPL when HCG levels were lowest and HPL highest are difficult to explain. Two analytes showing a correlation in the multiple regression analysis irrespective of which is considered the dependent variable may be regulated by a third factor. Similar stimulatory effects by Ca^{++} and inhibitory effects by Co^{++} on both HPL and HCG secretion by placental explants have been previously reported [23].

In evaluating correlation between growth factors and other reproductive hormones, we need to consider the inter-play of all such analytes known to change during pregnancy. We attempted this by studying most of the major hormones of pregnancy. Others have not simultaneously looked at this number of analytes. We selected oestradiol rather than oestriol, as the former is more potent though lower in concentration. We studied three major components of the insulin-like growth factor system, namely, IGF-I, IGF-II and IGFBP-1, the major IGF binding protein in the fetomaternal interface. Negative correlations between IGFBP-1 and prolactin, and oestradiol and IGF-II as well as positive correlations between prolactin and IGF-I and, progesterone and IGFBP-1 observed by us in vivo have not been reported previously. While some of these can be explained by known in vitro findings, why such relationships were limited to a particular period of gestation is not clear. A complex relationship exists between the IGF system and the placental hormones where components of the former appear to regulate or are regulated by some components of the latter. This is further modulated by changes in the endocrine milieu as pregnancy advances.

Acknowledgements

We thank the Director and Staff of the Castle Street Hospital for Women, Colombo and the study participants for their co-operation. This work was supported by

National Science Foundation (RG/2001/M/08), and a SAREC Grant for Molecular Biology and Biotechnology.

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