Serum levels of placental growth factor and retinol-binding protein-4 in pregnancy-induced hypertensive women

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Abstract: To investigate the relationship between clinical parameters of pre-eclampsia (PE) and serum levels of Retinol binding protein4 (RBP4) and Placental growth factor (PlGF). Patients and Methods: The study included 90 pregnant women categorized as Group I: Control group (n= 20), included pregnant women who continued their pregnancy without development of PE manifestations, Group II: included patients had Mild PE (n=56) and group III included patients had Severe PE (n=14). After clinical evaluation and ultrasonographic examination, samples of maternal peripheral blood were obtained either at time of diagnosis of PE in groups II and III or at time of delivery in control group for ELISA estimation of serum RBP4 and PlGF. Results: PE patients had significantly lower serum PlGF, but significantly higher serum RBP4 levels when compared to the corresponding levels of the control group. Serum levels of PlGF showed negative correlation with systolic and diastolic blood pressures (SBP and DBP) and extent of proteinuria, but showed positive significant correlation with birth weight, while serum levels of RBP4 showed positive significant correlation with DBP, extent of proteinuria and patients' body weight measures. Conclusions: RBP4 and PlGF were associated with the development and severity of PE.
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1. Introduction

Pregnancy is a physiological situation where major changes in energy homeostasis occur to meet the nutrient demands of fetal growth. The energy needs are met by increased food intake and/or mobilization of stored fuels and decreased sensitivity of maternal tissues to insulin. This change in insulin sensitivity allows for decreased glucose utilization and a shift to fat metabolism by maternal peripheral tissues and increased availability to the feto-placental unit. The pregnant state parallels the insulin-resistant states of obesity and type-2 diabetes, which are also characterized by insulin resistance, and can become manifested as gestational diabetes in humans (Herrera, 2000, Di Cianni et al., 2003).

Pre-eclampsia is the major cause of maternal and fetal morbidity and mortality, involving 15% to 20% of pregnancies in developed countries and even more in less developed parts of the world. Superficial placentation driven by immune maladaptation, with subsequently reduced concentrations of angiogenic growth factors and increased placental debris in the maternal circulation, are likely responsible (Dechend and Luft, 2008).

Retinol (vitamin A) bound to its specific transport protein, retinol-binding protein (RBP), is the predominant (95% or more) retinoid form in the fasting circulation. Postprandially, retinyl ester packaged in chylomicrons and chylomicron remnants can constitute a large percentage of the total retinoid present in the circulation. As RBP is the sole specific transport protein for retinol, it has been proposed to play an important role in the delivery of retinoid from mother to fetus. However, the mechanisms and the physiology of maternal-fetal vitamin A transfer are not fully understood (Soprano and Blaner, 1994, Sapin et al., 1998).

Dysregulation of maternal circulating adipokines has been implicated in several "great obstetrical syndromes" including pre-eclampsia, small-for-gestational age, neonate and fetal death. It has been suggested that adipokines provide a molecular link between metabolic derangements and inflammatory response in complicated pregnancies. Retinol binding protein 4, a novel adipokine, plays a role in obesity-related disorders, as well as in the regulation of the immune response (Vaisbuch et al., 2009). Solini et al., (2009) determined serum RBP4,

leptin, adiponectin, and resistin levels in hypertensive and normotensive lean non-pregnant women with normal glucose tolerance and found serum RBP4 levels are increased in naive hypertensive women and correlated with the degree of intima-media thickness

Placental growth factor is a pregnancy-specific hormone that has been proposed to play a role in trophoblast invasion and fetal growth, as well as maternal adaptation to pregnancy (Lacroix et al., 2005). PlGF demonstrates somatotrophic, lactogenic and lipolytic properties similar to pituitary growth hormone, although its growth-promoting activity surpasses its other functions. Syncytiotrophoblast and extravillous cytotrophoblast express PlGF mRNA and protein. This hormone is secreted in a non-pulsatile fashion and can be detected in maternal blood at as early as 5 weeks of gestation (Chellakooty et al., 2004) and increases throughout pregnancy until term (Chellakooty et al., 2002), at which time PlGF concentration has been observed to either plateau or slightly decrease (Chellakooty et al., 2004).

Central to the pathogenesis of PE is shallow placentation with abnormal maternal-placental vascular development. Shallow placentation causes release of endothelial deranging factors to the maternal circulation. Among these placenta-derived factors are the antiangiogenic proteins such as soluble fms-like tyrosine kinase receptor (sFlt1) which binds and reduces the free circulating levels of the proangiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). sFlt thereby blunts the beneficial effects of these proangiogenic factors on maternal endothelium, with consequent maternal hypertension and proteinuria (Redman and Sargent, 2005, Maynard et al., 2003, Troisi et al., 2008).

The aim of the present study is to evaluate the association between the levels of serum RBP4 and PIGF and the development of PIH or its severity.

2. Patients and Methods

Pre-eclampsia was diagnosed by the presence of gestational hypertension beginning after the 20th week of pregnancy with an absolute blood pressure ≥140 mmHg systolic and/or 90 mmHg diastolic on at least two occasions, 4 hours apart, and proteinuria (one dipstick measurement ≥2+ on a voided random urine sample). Severe PE was defined as severe hypertension (diastolic blood pressure ≥ 110 mmHg) plus mild proteinuria (2+ protein by dipstick measurement) or mild hypertension plus severe proteinuria (≥ 3+ protein by dipstick measurement). Mild PE was defined as mild hypertension with diastolic blood pressure <110 mmHg plus mild proteinuria (≥ 2+ protein by dipstick measurement). Patients with eclampsia were also classified in the severe pre-eclampsia category (Gifford et al., 2000).

This study was conducted at Benha University hospital in conjunction with Medical Biochemistry Department, Benha Faculty of Medicine and included 90 pregnant women, signed a fully informed written consent and categorized as Group I: Control group (n= 20), included pregnant women who continued their pregnancy without development of PE manifestations, group II: included patients had Mild PE (n=56) and group III included patients had Severe PE (n=14). Exclusion criteria included multiple gestation and preexisting medical conditions such as diabetes, chronic hypertension, and renal diseases.

At time of enrollment in the study, all women underwent full history taking, general and abdominal examination to determine a baseline arterial blood pressure and body mass index (BMI). Ultrasonographic examination was conducted to confirm the gestational age, and to exclude the presence of fetal congenital abnormalities. Body mass index was calculated according to the equation: BMI = [(Weight)/ (Height) ^{2}]. A person with a BMI of ≥30 are considered obese and morbidity rise sharply when the BMI is >30 kg/m^{2} (Vella et al., 2003).

Throughout the period since baseline data collection, all women were examined weekly for the progress of pregnancy and fetal wellbeing, the extent of hypertension and occurrence of other complications. Urine analysis was performed for the presence of urinary tract infection and the degree of proteinuria.

Samples of maternal peripheral blood were obtained either at time of diagnosis of pre-eclampsia in hypertensive group or at time of delivery in control group. Collected maternal blood samples were allowed to clot then serum was separated by centrifugation at 2000 rpm for 10 min. Serum was removed, placed in pyrogen-free Eppendorf tubes and stored at -80°C until ELISA assayed (within one month) for estimation of serum RBP4 (AdipoGen Inc., Seoul, Korea), (Lewis et al., 2007) and PlGF (RayBiotech, USA) concentrations, (Nemzek et al., 2001).

Statistical analysis: obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using one-way ANOVA test and Chi-square test. Statistical analysis was conducted using the SPSS (Version 10, 2002) for Windows statistical package. P value <0.05 was considered statistically significant.

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3. Results

The study was extended till enrollment of 70 PE pregnant women; Table (1) shows that mean age of enrolled PE patients was 27.9±3; range with a mean BMI of 31.2±1.8 kg/m^2. At time of diagnosis, mean SBP was 150.3±5.9 mmHg and mean DBP was 100.6±6.4 mmHg. All patients were proteinuric with a mean level of 1.8±0.7; range by dipstick measurement. Mean gestational age at time of enrollment was 30.9±3.8 weeks. The study also included a control group consisted of 20 pregnant women of cross-matched age and gestational age.

Table (1): Characteristics of the entire study population at time of enrollment

		Control group (n=20)	PE group (n=70)
Age (years)		28.3±2.4 (25-33)	27.9±3 (22-33)
Weight (Kg)		80.5±2.9 (75-85)	82.9±2.9 (79-90)
Height (cm)		164.7±4.9 (158-172)	163±3.5 (156-173)
BMI (kg/m^2)		29.7±2.1 (27.3-33.6)	31.2±1.8 (27.4-36.1)
Nulli:multipara		13:7	43:27
Gestational age (wk) at time of enrollment		29.7±3.6 (24-36)	30.9±3.8 (23-37)
Blood pressure	SBP	122±4.7 (115-130)	150.3±5.9 (140-165)*
	DBP	79.3±5.2 (70-85)	100.6±6.4 (90-118)*
‡Level of protein in urine		0.7±0.5 (0 to +1)	1.8±0.7 (+1 to +4)*

Data are presented as mean±SD, ranges are in parenthesis
BMI: Body mass index
SBP: systolic blood pressure
DBP: diastolic blood pressure
*: significant difference versus control group
‡: Level of protein in urine as judged by dipstick measurement and expressed as number of + marks

Table (2) shows that 14 women had severe pre-eclampsia with a mean SBP and DBP of 154.8±8.5 mmHg and 107.9±7.4 mmHg, respectively and the mean proteinuria level of 2.7±0.8 by dipstick measurement. While the other 56 women had mild pre-eclampsia with a mean SBP and DBP of 149.2±4.4 mmHg and 98.8±4.7 mmHg, respectively and mean proteinuria level of 1.6±0.5 by dipstick measurement. Patients developed severe PE had significantly higher mean blood pressure measures and significantly higher level of protein in urine compared to those had mild PE. However, other evaluated parameters showed non-significant difference between severe and mild PE patients.

Table (2): Clinical characteristics of pre-eclamptic patients

		Mild PE (n=56)	Severe PE (n=14)
Age (years)		27.8±3.2 (23-33)	27.9±3 (22-33)
Weight (Kg		82.9±3.2 (79-87)	82.8±2.8 (79-90)
Height (cm)		164±2.8 (159-169)	162.7±3.7 (156-172)
BMI (kg/m^2)		30.9±1.3 (29-33.2)	31.3±1.9 (27.4-36.1)
Nulli: multipara		35:21	8:6
Gestational age (wk) at time of enrollment		30.7±4.2 (24-36)	30.9±3.8 (23-37)
Blood pressure	SBP	149.2±4.4 (140-155)	154.8±8.5 (142-165)*
	DBP	98.8±4.7 (90-106)	107.9±7.4 (95-118)*
‡Level of protein in urine		1.6±0.5 (+1 to +2)	2.7±0.8 (+2 to+4)*

Data are presented as mean±SD, ranges are in parenthesis
BMI: Body mass index
SBP: systolic blood pressure
DBP: diastolic blood pressure
*: significant difference versus control group
‡: Level of protein in urine as judged by dipstick measurement and expressed as number of + marks

Table (3) shows that among the studied 90 women, 56 women (62.2%) had vaginal delivery, while 34 women (37.8%) had cesarean section (CS). Women developed PE had significantly higher, (X^2=3.853, p<0.05) frequency of CS compared to control group (41.4% versus 25%, respectively) and women developed severe PE had significantly higher, (X^2=4.962, p<0.01) frequency of CS compared to control group (57.1% versus 25%, respectively). Gestational age at time of delivery was significantly shorter in PE group compared to control group and in those developed severe PE compared to those had mild PE. Moreover,

birth weight was significantly (P<0.01) lower in PE group compared to control group and in those developed severe PE compared to those had mild PE.

Table (3): Delivery data of the study population

		Control (n=20)	Mild PE (n=56)	Severe PE (n=14)
‡GA (weeks)		39.7±1.3 (38-42)	35.4±1.3 (34-38)	33.3±1.1† (32-35)
Gestational weight (gm)		3.31±0.5 (2.5-4.2)	2.8±0.62 (1.6-3.9)	2.44±0.55† (1.6-3.35)
Delivery mode	CS	5 (25%)	21 (37.5%)	8 (57.1%)
	Vaginal	15 (75%)	35 (62.5%)	6 (42.9%)

Data are presented as mean±SD & numbers, ranges & percentages are in parenthesis
CS: cesarean section
‡Gestational age at time of delivery
*: significant difference versus control
†: significant difference versus mild PE

Table (4) shows significantly lower PlGF and higher RBP4 serum levels in PE women compared to control women and in women had severe PE compared to both controls and those had mild PE with significant difference between mild pre-eclamptic and controls.

Table (4): Mean (±SD) levels of serum PlGF and RBP4 in control and PE groups

		Serum PlGF (µg/ml)	Serum RBP4 (ng/ml)
Control group (n=20)		346.7±113.6 (221.5-578.6)	20.7±8.6 (9-45)
PE group	Mild (n=56)	242.3±84.3* (103.7-395.4)	60.1±18.4* (29-105)
	Severe (n=14)	183±42.1*† (122.4-250.4)	104.9±17*† (72-127)
	Total (n=70)	230.4±81* (103.7-395.4)	69.1±25.5* (29-127)

Data are presented as mean±SD, ranges are in parenthesis
*: significant difference versus control
†: significant difference versus mild pre-eclampsia

Figure (1) shows a significantly lower PlGF serum levels in PE women compared to control women, with significantly lower levels in women had severe PE compared to controls and those had mild PE and between patients had mild PE and controls. On contrary, figure (2) shows a significantly higher RBP4 serum levels in PE women compared to control women with significantly higher levels in women had severe PE compared to controls and those had mild PE and between patients had mild PE and controls.

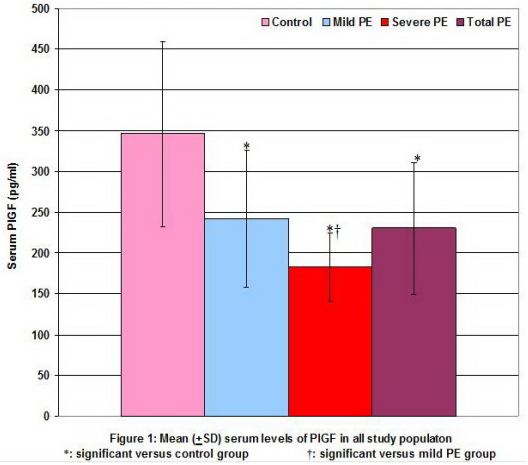

Figure 1: Mean (±SD) serum levels of PlGF in all study populaton
*: significant versus control group †: significant versus mild PE group

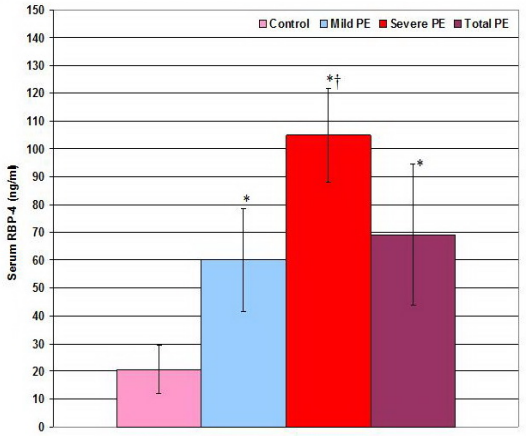

Figure 2: Mean (±SD) serum levels of RBP-4 in all study populaton
*: significant versus control group †: significant versus mild PE group

Serum levels of PlGF showed negative significant correlation with the severity of PE manifested as SBP (-0.429, p<0.001), DBP (-0.276, p=0.021) and the extent of proteinuria (-0.236, p<0.001), while serum levels of RBP4 showed positive significant correlation with as DBP (0.509, p<0.001) and extent of proteinuria (0.417, p<0.001). Serum levels of PlGF showed positive significant correlation with birth weight (0.312, p=0.009). On contrary, such correlation was non-significant with serum RBP4 that showed positive significant correlation with body weight (0.246, p=0.040) and mass index (0.318, p=0.007). Moreover, there was a

negative non-significant correlation between serum levels of PlGF and RBP-4.

4. Discussion

Depending on both DBP and extent of proteinuria, 14 cases had developed severe PE and 56 cases had mild PE. All cases developed PE, irrespective of its severity, showed significantly higher serum levels of RBP4 and lower PlGF compared to levels estimated in control group and in patients who had developed severe PE compared to those had mild PE. These findings pointed to an association between both RBP4 and PIGF serum levels and the development of PE. In support of such assumption, there was a significant correlation between serum levels of both parameters and estimated DBP and extent of proteinuria defined at time of inclusion in the study.

As regards serum RBP4, the obtained results go hand in hand with Inoue et al., (2009) who found that serum RBP4 levels were increased in pregnant women with PIH compared with normal pregnancies. However, Stepan et al., (2009) found that the mean maternal serum RBP4 concentrations were not significantly different in PE as compared with controls. This discrepancy could be attributed to small sized sample of PE women (n=16) studied by Stepan et al., (2009). In support our results, Vaisbuch et al., (2009) found that the maternal plasma RBP4 concentration was higher among patients with PE than in those with a normal pregnancy, and in patients with preterm PE (<37 weeks) than those with either term PE or normal pregnancy.

The obtained data suggested a participation of RBP4; an adipocytokine; in the pathogenesis and/or modulation of the PIH process and pointed to a role exerted by the adipose tissue as an endocrine organ. In support of such assumption, the current study reported a positive significant correlation between serum RBP4 and body weight and body mass index which coincided with that reported by Broch et al. (2009) who found that circulating RBP4 levels positively correlate with waist circumference, one of the obesity-related parameters.

The role played by RBP4 in pathogenesis and/or modulation of PIH could be attributed to concomitant insulin resistance that occurs during pregnancy and several previous studies documented such attribution between RBP4 and insulin resistance; Yang et al. (2005) demonstrated that adipose tissue-specific (Glut4)knockout mice have increased serum levels of RBP4 and downregulation of GLUT4 in adipose tissue is an important feature of insulin resistance. Clinical studies also reported that RBP4 is positively related to insulin resistance especially in obese (Graham et al., 2006) patients with impaired glucose tolerance (Cho et al., 2006) and type-2 diabetics (Cheng et al., 2009).

Insulin has important endothelial-dependent vasodilator actions mediated by nitric oxide (NO) via phosphatidylinositol 3-kinase (PI 3-kinase)–dependent activation of endothelial NO synthase (Zeng et al., 2000), but interestingly, insulin also has vasoconstrictor actions mediated by mitogen-activated protein kinase (MAPK)-dependent endothelial secretion of endothelin-1 (ET-1) (Cardillo et al., 1999). Insulin resistance is characterized by selective impairment in PI 3-kinase–dependent signaling pathways regulating metabolic actions of insulin in skeletal muscle with intact MAPK signaling pathways (Cusi et al., 2000) and is accompanied by compensatory hyperinsulinemia that serves to overcome impairment in PI 3-kinase signaling to maintain euglycemia. However, this hyperinsulinemia is predicted to overdrive unaffected MAPK signaling that may promote pathological actions of insulin, including increased secretion of ET-1, increased expression of vascular adhesion molecules, proliferation of vascular smooth muscle, increased expression of proinflammatory cytokines and activation of cation pumps (Potenza et al., 2005). These factors may shift the balance between vasodilator and vasoconstrictor actions of insulin and result in predisposition to hypertension in insulin-resistant states (Sowers, 2004).

As another explanation for association between RBP4 and PIH, Cabré et al., (2007) found that plasma RBP4 concentration might be a biomarker of nephropathy and cardiovascular disease in type 2 diabetic subjects and Frey et al., (2009) also demonstrated that there is a strong correlation between kidney function and RBP4 isoforms; thus serum RBP4 may reflect the degree of renal affection in these pre-eclamptic women and in support of this, the our study reported a positive significant correlation between serum RBP4 and extent of proteinuria.

Our results also showed that serum PlGF levels were significantly lower in PE group especially patients who had severe PE, a fact indicating abnormal placentation process as PlGF; an angiogentic factor; is physiologically essential for embryogenesis and development.

Such decrease of serum PlGF levels was manifested as increased frequency of small-for-gestational age (SGA) newborns with a negative significant correlation between serum PlGF and birth weight. These findings go hand in hand with multiple previous studies which reported significantly lower serum PlGF in association with low birth weight in pre-eclamptic patients in comparison to control group (Shibata et al., 2005, Espinoza et al., 2007, Romero et al., 2008).

Osol et al., (2008) experimentally reported that PlGF is a potent vasodilator of several vessel types in both humans and rats. Its potency and mechanism vary with physiological state and vessel location and are mediated solely by the vascular endothelial growth factor receptor-1 (VEGFR-1) subtype and gestational changes in the uterine circulation. The authors added that this might suggest that PIGF may play a role in modulating uterine vascular remodeling and blood flow during the pregnant state.

Erez et al., (2008) documented that changes in the maternal plasma concentrations of soluble form of endoglin (s-Eng), sVEGFR-1, PlGF or their ratios between the first and second trimesters of pregnancy confer an increased risk to deliver an SGA neonate and/or develop PE. Thus the effect imposed by decreased serum PlGF and development or maintenance of PE could be attributed to the loss of the vasodilator effect of PlGF. In support of this attribution, the current study reported a negative significant correlation between serum PlGF and both SBP and DBP.

In conclusion: both RBP4 and PlGF were strongly associated with the development and/or severity of PE. However, such association might need wider scale studies to be confirmed.

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