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Maternal Plasma VEGF, sVEGF-R1, and P*l*GF Concentrations in Preeclamptic and Normotensive Pregnant Zimbabwean Women

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Summary

Vascular endothelial growth factor (VEGF), a disulphide-linked homodimeric glycoprotein that is selectively mitogenic for endothelial cells, plays an important role in vasculogenesis and angiogenesis. Preeclampsia, a relatively common complication of pregnancy that is characterized by diffuse endothelial dysfunction possibly secondary to impaired trophoblast invasion of the spiral arteries during implantation, has recently been associated with alterations in maternal serum/plasma concentrations of VEGF, and other related growth factors and their receptors. We examined the relationship of maternal plasma VEGF, sVEGF-R1 and P*l*GF levels to the risk of preeclampsia among women delivering at Harare Maternity Hospital, Zimbabwe**.** 131 pregnant women with preeclampsia and 175 controls were included in a case-control study. Maternal plasma concentrations of each biomarker were measured using enzymatic methods. We used logistic regression to calculate odds ratios (OR) and 95 % confidence intervals (CI). Preeclampsia risk was inversely related with quartiles of plasma VEGF (OR: 1.0, 1.0, 0.7, and 0.5, with the lowest quartile as reference; p for trend = 0.06). We noted a strong positive association between preeclampsia risk and sVEGF-R1 concentrations (OR: 1.0, 6.5, 9.7, 31.6, with the first quartile as the referent group; p for trend < 0.001). After adjusting for confounders, we noted that women with sVEGF-R1 concentrations in the highest quartile (≥ 496 pg/ml), as compared with those in the lowest quartile (< 62 pg/ml) had a 31.6-fold increased risk of preeclampsia (OR = 31.6, 95 % CI 7.7-128.9). There was no clear evidence of a linear relation in risk of preeclampsia with P*l*GF concentrations. In conclusion, plasma VEGF, sVEGF-R1 and P*l*GF concentrations (measured at delivery) were altered among Zimbabwean women with preeclampsia as compared with normotensive women. Our results are consistent with some, though not all, previous reports. Prospective studies are needed to: 1) identify modifiable determinants of maternal plasma concentrations VEGF, sVEGF-R1, and P*l*GF; and 2) evaluate the temporal relationship between observed alterations of these biological markers in preeclamptic pregnancies.

Key words
VEGF • sVEGF-R1 • PlGF • Preeclampsia • Pregnancy • Risk Factors

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Introduction

Preeclampsia, a life-threatening complication of pregnancy, is characterized by the onset of high blood pressure and proteinuria. Undoubtedly the placenta is involved in the pathogenesis of preeclampsia since the syndrome is eradicated with the termination of pregnancy. The pathogenesis of preeclampsia is thought to involve three components: defective placentation, placental ischemia, and endothelial cell dysfunction (angiogenesis) leading to complications at the placental vascular level (Roberts and Lain 2002). Angiogenic syndrome may initiate as placental factors enter maternal circulation eventually causing endothelial cell dysfunction which leads to hypertension and proteinuria (Page *et al.* 2000, Maynard *et al.* 2003).

Angiogenesis is rare in adults with exceptions of the female reproductive tract and some pathological conditions (Folkman and Shing 1992, Folkman 1995, Gordon *et al.* 1995). Mammalian placenta requires extensive angiogenesis to establish a suitable vascular network for the supply of oxygen and nutrients to the fetus (King 1987). A variety of angiogenic growth factors from the vascular endothelial growth factors family such as vascular endothelial growth factor (VEGF), placental growth factor (P*l*GF) and soluble vascular endothelial growth factor receptor 1 (sVEGF-R1) are expressed in the placenta (Park *et al.* 1994, Cooper *et al.* 1995, Breier *et al.* 1995, Dumont *et al.* 1995, Vuckoviv *et al.* 1996).

Vascular endothelial growth factor (VEGF) (Ferrara and Henzel 1989), also known as vascular permeability factor (VPF) (Senger *et al.* 1983) is a homodimeric 34-42 KDa, heparin-binding glycoprotein with potent angiogenic, mitogenic and vascular permeability-enhancing activities specific for endothelial cells. The amino acid sequence of VEGF exhibits primary structural homology to the A and B chain of platelet-derived growth factor (PDGF). A cDNA encoding a protein having 53 % amino sequence homology in the PDFG-like region of VEGF has been isolated from a human placental cDNA library (Maglione *et al.* 1991). This protein, named placental growth factor (P*l*GF) is now recognized to be a member of the VEGF family of growth factors (Schott and Morrow 1993, Ferrara *et al.* 1992). VEGF expression has been found in activated macrophages (Fava *et al.* 1994), keratinocytes (Brown *et al.* 1992a), renal glomerular visceral epithelium (Brown *et al.* 1992b), hepatocytes (Monacci *et al.* 1993), aortic smooth muscle cells (Ferrara *et al.* 1991) and embryonic fibroblasts (Breier *et al.* 1992). In contrast to the widespread distribution of VEGF, the expression of P*l*GF is limited to placental tissue, choriocarcinoma cells and cultured endothelial cells (Maglione *et al.* 1991, 1993 Hauser and Weich 1993).

Both P*l*GF and VEGF have been shown to be equipotent in stimulating tissue factor production and chemotaxis in monocytes (Clauss *et al.* 1996, Barleon *et al.* 1996). In comparison to VEGF, P*l*GF is a weak endothelial cell mitogen and chemoattractant (Park *et al.* 1994, Cao *et al.* 1996). The VEGF/P*l*GF heterodimer has been shown to promote capillary growth *in vivo* and to chemoattract endothelial cells *in vitro*; VEGF/P*l*GF heterodimers also exhibit intermediate potency as an endothelial mitogen relative to the homodimeric forms of VEGF and P*l*GF (Cao *et al.* 1996, DiSalvo *et al.* 1995, Clauss *et al.* 1996, Barleon *et al.* 1996, Park *et al.* 1994).

The biological effects of the VEGF family members are mediated by members of the class III subfamily of receptor tyrosine kinases (RTKs). These contain seven immunoglobulin-like repeats in their extracellular domains (Mustonen and Alitalo 1995). At least three RTKs that bind various VEGF family members have been cloned, VEGF-R1 (Flt-1), VEGF-R2 (KDR/Flt-1), and VEGF-R3 (Flt-4) (Shibuya *et al.* 1990, Pajusola *et al.* 1992, Seetharam *et al.* 1995, Sawano *et al.* 1996). The human VEGF-R1 was originally discovered through the screening of a human placental cDNA library (Shibuya *et al.* 1990). P*l*GF has been shown to bind VEGF-R1 but not VEGF-R2 and VEGF-R3, with high affinity (Sawano *et al.* 1996). VEGF has been shown to bind both VEGF-R1 and VEGF-R2, but not VEGF-R3, with high affinity (Mustonen and Alitalo 1995, Joukov *et al.* 1996). A soluble form of VEGF-R1 (sVEGF-R1) has also been identified in culture medium conditioned by the growth of human umbilical vein endothelial cells (HUVECs); that is accomplished through an alternate splicing at the pre-mRNA level (Kendall and Thomas 1993).

In this manuscript we examine the relationship among VEGF, sVEGF-R1 and P*l*GF and the risk of preeclampsia in a case-control study on plasma samples and clinical information from Sub-Saharan, African women.

Material and Methods

Subjects for this analysis were recruited between June 1995 and April 1996 as part of a case-control study

designed to study the epidemiology of preeclampsia and eclampsia among Zimbabwean women. Details regarding data collection methods have been previously described (Williams *et al.* 1998, 2003). During the study period, women diagnosed with eclampsia, preeclampsia, and normotensive women (controls) were recruited from the Labor and Delivery ward of the Harare Maternity Hospital, a University of Zimbabwe Medical School affiliated hospital. All 200 eligible cases that were approached agreed to participate in the study. Cases were comprised of 37 patients with eclampsia and 163 patients with preeclampsia. Of the 201 controls approached, 200 agreed to participate in the study. The Medical Research Council of Zimbabwe and the Human Subjects Committee of the University of Washington Medical Center approved this investigation. All participants provided informed consent.

From the original study population of 163 women with preeclampsia (according to the then-current ACOG 1996 diagnostic criteria) (ACOG 1996), we selected 131 cases (80 %) that had adequate amounts of plasma samples available for laboratory analyses. Preeclampsia was defined as persistent (6 or more hours) blood pressure of at least 140/90 mm Hg. Proteinuria was defined as urine protein concentration ≥ 30 mg/dl or more (or 1+ on a urine dipstick) in at least two random specimens collected at least 4 hours apart. Approximately 97 % (157 of 180 normotensive subjects) with available plasma samples were available for inclusion in this analysis. There were too few eclampsia cases to study the relation between maternal plasma VEGF, sVEGF-R1 and P*l*GF concentrations and eclampsia risk. In addition, since magnesium which is used for management of eclampsia, has been shown to influence angiogenesis through regulation of VEGF (Lapidos *et al.* 2001), we excluded eclampsia cases from this analysis.

A structured interview questionnaire, administered during participants' postpartum hospital stay, was used to collect information on maternal sociodemographic, medical, reproductive and life style characteristics during in-person interviews. Maternal and infant records were reviewed to collect detailed information concerning antepartum, labor and delivery characteristics as well as conditions of the newborn. Maternal anthropometric measures (height, weight and mid-arm circumference) were taken during participants' postpartum hospital stay. Maternal non-fasting, blood samples, collected after delivery in EDTA-containing blood collection tubes, were immediately processed where plasma was separated in a refrigerated centrifuge. Aliquots of maternal plasma were stored at –70 °C until analysis. VEGF, sVEGF-R1, and P*l*GF concentrations were determined using enzyme-linked immunosorbent assay (ELISA) procedures, reagents and standards according to manufacturer specifications (R&D Systems, Minneapolis, MN). According to the manufacturer, the minimal detectable concentrations for sVEGF-R1, P*l*GF and VEGF were 5, 7 and 5 pg/ml, respectively. For all the assays, the intra- and inter-assay coefficients of variation were less than 8 %. All laboratory assays were performed without knowledge of the case or control status.

Frequency distributions of maternal sociodemographic characteristics, medical and reproductive histories according to each case and control status were examined. Student's t-test was used to test mean differences in maternal plasma concentrations of each analyte between cases and controls. To estimate the relative association between preeclampsia and concentrations of plasma analytes, we categorized each subject according to quartiles determined by their distribution in normotensive control subjects. Using the lowest quartile category of each analyte (e.g. VEGF) for the reference group, odds ratios (OR) and 95 % confidence intervals (CI) were estimated for each of the remaining three quartiles. Logistic regression procedures were used to calculate maximum likelihood estimates for the coefficients and their standard errors were used for calculating the odds ratios and 95 % confidence intervals, adjusted for confounders (Rothman and Greenland 1998). In multiple logistic regression models, the significance for a monotonic trend was assessed by treating the four quartiles as a continuous variable after assigning a score (e.g. 1, 2, 3, 4 for each successive quartile) as its value. To avoid confusion, we entered variables into a logistic regression model one at a time. We then compared the adjusted and unadjusted ORs. Final logistic regression models included covariates that altered unadjusted ORs by at least 10 %, as well as maternal age and parity. We also explored the possibility of a nonlinear relation between each plasma analyte and preeclampsia risk using generalized additive modeling (GAM) procedures (Hastie and Tibshirani 1990) by using S-PLUS (version 6.1, Insightful Corp 2002). All other analyses were performed using Stata 7.0 statistical software (Stata, College Station, TX). All reported p-values are two-tailed.

Table 1. Distribution of preeclampsia cases and normotensive control subjects according to selected characteristics (Harare, Zimbabwe 1995-1996).

Characteristic	Preeclampsia Cases (n = 131) n	%	Control Subjects (n = 175) n	%
Maternal age (years)				
< 19	11	8.4	25	14.3[f]
19-34	101	77.1	139	79.4
≥35	19	14.5	11	6.3
Maternal age (years)[*]	25.6 ± 0.6		24.5 ± 0.4[f]	
Unmarried	14	10.7	25	14.3
Nulliparous	67	51.2	107	61.1
Previous miscarriage	13	9.9	16	9.1
Previous stillbirth	12	9.2	7	4.0[f]
Maternal body mass index (kg/m^2)[*]	27.4 ± 0.4		25.2 ± 0.3[f]	
Maternal body mass index (kg/m^2)[*]				
<19.9	0	0.0	3	1.7[f]
20-24.9	46	35.1	92	52.6
25-29.9	47	35.9	63	36.0
≥30	35	26.7	15	8.6
Missing	3	2.3	2	1.1
Mid-arm circumference (cm)[*]	27.0 ± 0.3		25.2 ± 0.2[f]	
Gestational age at delivery (weeks)[*]	36.4 ± 0.3		38.5 ± 0.2[f]	

[*]Mean ± standard error of mean (S.E.M.). [f]p-value < 0.05

Table 2. Plasma VEGF, PlGF and sVEGF-R1 and (pg/ml) concentrations among preeclamptic and normotensive pregnant women (Harare, Zimbabwe, 1995-1996).

Biomarker (pg/ml)	Preeclampsia Cases (n=131) Median [IQR[*]]	Control Subjects (n=175) Median [IQR]	Mann-Whitney U test P-value[*]
VEGF	10.9 [6.1 – 20.1]	13.6 [6.4 – 23.0]	0.09
VEGF-R[**]	636.4 [302.8 – 1349.4]	222.8 [62.0 – 495.9]	< 0.001
PlGF[***]	12.4 [8.9 – 20.9]	12.1 [7.2 – 25.8]	0.60
Ratio of VEGF-R/ PlGF [***]	46.0 [21.0 – 87.5]	15.8 [7.6 – 33.5]	< 0.001

[*]IQR = Intra-quartile range. [**] Analysis limited to 128 cases and 173 controls. [***] Analysis limited to 127 cases and 167 controls.

Results

Sociodemographic and medical characteristics of the study participants are presented in Table 1. Median VEGF concentrations tended to be lower in preeclamptic as compared with controls (Table 2). Median sVEGF-R1 concentrations were higher in cases than in controls (p<0.001). 38.9 % of preeclampsia cases were at or above the median plasma concentration for VEGF. 80.9 % of cases were at or above the median plasma concentration for sVEGF-R1. No statistical difference in the median plasma P*l*GF concentration was noted for preeclampsia cases as compared with normotensive controls.

We evaluated the risk of preeclampsia with varying concentrations of maternal plasma VEGF, P*l*FG, and sVEGF-R1 (Table 3). There was evidence of a statistically significant inverse relation between maternal plasma VEGF concentrations and risk of preeclampsia. After adjusting for maternal age, nulliparity, adiposity, and gestational age at blood collection, women in the

highest quartile for VEGF (≥ 23.0 pg/ml) had a 50 % reduced risk of preeclampsia as compared with women in the lowest quartile (< 26.5 pg/ml) (adjusted OR = 0.5; 95 % CI 0.2-1.1), though this association did not reach statistical significance.

Table 3. Odds ratios (OR) and 95 % confidence intervals (CI) of preeclampsia according to quartile of maternal plasma VEGF, sVEGF-R1 and P*l*GF concentrations, Harare, Zimbabwe, 1995-1996.

Biomarker Concentrations	**Preeclampsia Cases** n	**Subjects** n	**Control Unadjusted OR (95 % CI)**	**Adjusted OR (95 % CI)†**
VEGF (pg/ml)				
Q1 < 6.5	34	44	1.0 (referent)	1.0 (referent)
Q2 6.5 – 13.5	46	44	1.4 (0.7 - 2.5)	1.0 (0.5 - 2.0)
Q3 13.6 – 22.9	27	44	0.8 (0.4 - 1.5)	0.7 (0.3 - 1.4)
Q4 ≥ 23.0	24	43	0.7 (0.4 - 1.4)	0.5 (0.2 - 1.1)
P-value for linear trend			0.16	0.06
sVEGF-R1 (pg/ml)				
Q1 < 62.0	3	43	1.0 (referent)	1.0 (referent)
Q2 62.0 – 222.8	19	44	6.2 (1.7 - 22.4)	6.5 (1.5 - 28.1)
Q3 222.9 – 495.9	32	43	10.7 (3.0 - 37.5)	9.7 (2.3 - 40.6)
Q4 ≥ 496.0	74	43	24.7 (7.2 - 84.3)	31.6 (7.7 - 128.9)
P-value for linear trend			<0.001	<0.001
Q1 < 62.0	3	43	1.0 (referent)	1.0 (referent)
Decile 10 ≥ 874.4	49	18	39.0 (10.8 - 141.6)	56.7 (11.0 - 293.2)
P*l*GF (pg/ml)				
Q1 < 7.3	16	42	1.0 (referent)	1.0 (referent)
Q2 7.3 – 12.0	45	41	2.9 (1.4 – 5.9)	2.3 (1.0 – 5.1)
Q3 12.1 – 25.8	43	43	2.6 (1.3 – 5.4)	1.8 (0.8 – 4.1)
Q4 ≥ 25.9	23	41	1.5 (0.7 - 3.2)	1.3 (0.6 – 3.0)
P-value for linear trend			0.53	0.85
Ratio of sVEGF-R1/ P*l*GF				
Q1 < 7.6	4	42	1.0 (referent)	1.0 (referent)
Q2 7.6 – 15.7	17	41	4.4 (1.3 - 14.0)	4.4 (1.2 - 16.8)
Q3 15.8 – 33.4	30	43	7.3 (2.4 - 22.6)	8.9 (2.4 - 32.4)
Q4 ≥ 33.5	76	41	19.5 (6.5 - 58.1)	24.9 (7.1 - 87.1)
P-value for linear trend			<0.001	<0.001
Q1 < 7.6	4	42	1.0 (referent)	1.0 (referent)
Decile 10 ≥ 68.0	39	16	25.6 (7.9 - 83.2)	35.4 (7.5 - 166.2)

† Adjusted for maternal age (continuous), nulliparity (yes/no), maternal adiposity, mid-arm circumference (continuous), and gestational age (continuous).

There was a very strong positive relation between plasma sVEGF-R1 and preeclampsia risk (adjusted p-value for linear trend in risk < 0.001). The odds ratio for suggestively higher quartiles with the lowest quartile (< 62.0 pg/ml) as the referent group, were as follows: 6.5, 9.7, and 31.6. Women in the highest quartile (≥ 496.0 pg/ml) had a 31.6-fold increased risk of preeclampsia as compared with women in the lowest

quartile (adjusted 95 % CI 7.7-128.9). To further evaluate the association between preeclampsia risk and extremely high plasma sVEGF-R1 concentrations, we identified those study subjects with concentrations falling in the highest decile of the control distribution (i.e. 49 cases and 18 controls). For this analysis, women with VEGF concentrations in the lowest quartile were used as the referent group. Women with extremely high plasma sVEGF-R1 concentrations (≥ 874.4 pg/ml) had a 56.7-fold increased risk of preeclampsia (adjusted OR = 56.7; 95 % CI 11.0-293.2) as compared with women with concentrations in the lowest quartile.

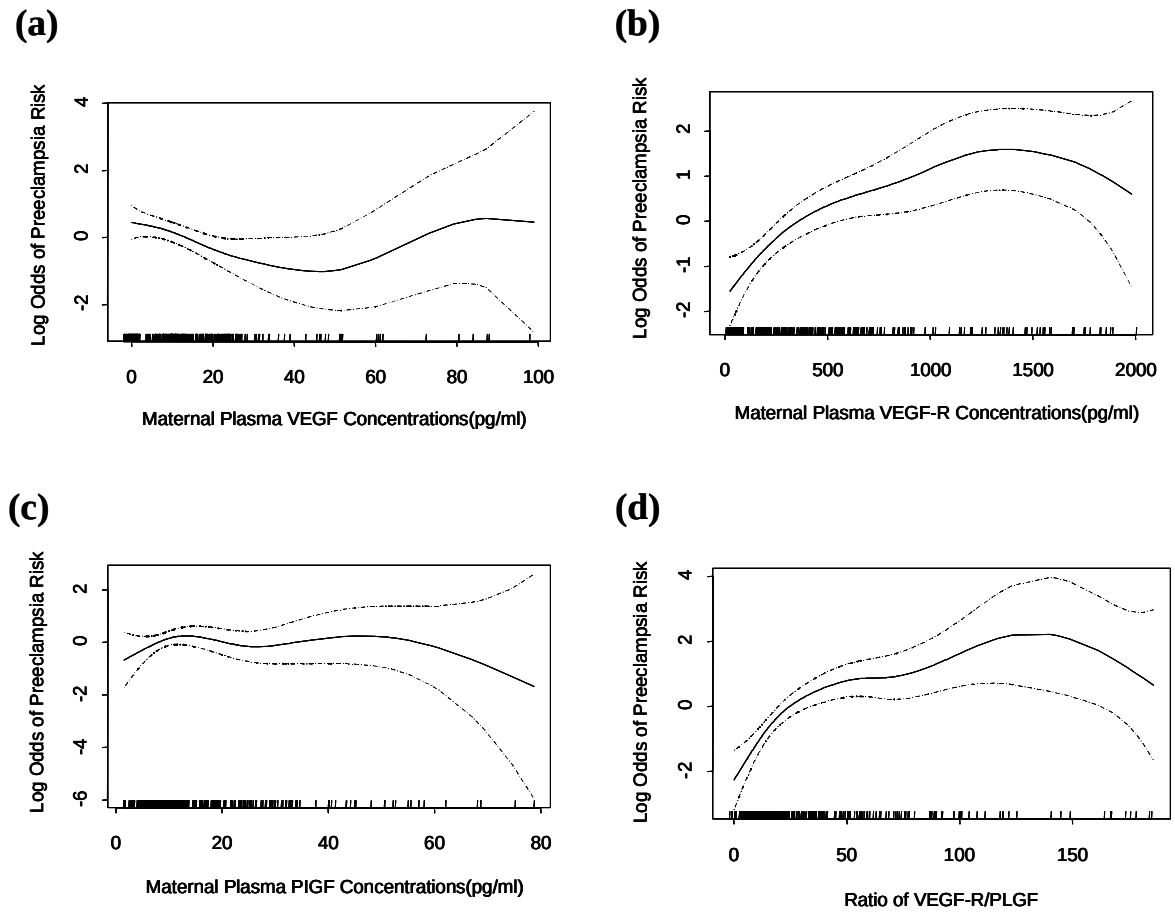

Fig. 1. Relationship between maternal plasma analytes, measured in postpartum blood samples and the log-odds of risk of preeclampsia (solid line), with 95 % confidence interval (dotted lines) for VEGF (*a*), sVEGF-R1 (b), PlGF (c), and sVEGF-R1/ PlGF ratio (d).

There was no clear pattern of a linear relation between the increased risk of preeclampsia with increasing concentrations of P*l*GF. Rather, visual inspection of the adjusted odds ratios (Table 3) suggested that the shape of the relation between preeclampsia risk and P*l*GF concentrations may be an inverted U-shaped. The adjusted odds ratios for each of the successive quartiles (lowest to highest were as follows): 1.0 (referent), 2.3, 1.8 and 1.3. The p-value for a test of linear trend was 0.85, which is consistent with the absence of a linear trend in proportion of preeclampsia risk with maternal plasma P*l*GF concentrations.

As expected, when we assessed the ratio of maternal plasma sVEGF-R1 and P*l*GF concentrations, we found evidence of a strong positive relation between the ratio and preeclampsia risk (Table 3, bottom panel). The highest preeclampsia risk was found among those with ratio values in the top decile versus those in the lowest decile (adjusted OR = 35.4; 95 % CI 7.5-166.2).

We next sought to graphically explore the shape of the relation of preeclampsia risk in relation to each analyte. We modeled the risk of preeclampsia in relation to maternal plasma concentrations of each analyte expressed as a continuous variable using a generalized additive model (GAM). From these analyses, we noted an approximately inverse linear relation between the log-

odds of preeclampsia and plasma VEGF concentrations above 50 pg/ml (Fig. 1a). There were too few subjects with higher plasma VEGF concentrations to explore the shape of the relation with any reasonable precision. We also noted a very strong monotonic increase in the log-odds of preeclampsia risk with increasing concentrations of maternal plasma sVEGF-R1 (Fig. 1b). The shape of the curve appears to plateau beginning with concentrations above 1000 pg/ml. However, the numbers of subjects at that end of the curve was again low and 95 % confidence intervals are wide, reflecting limited precision in this part of the curve.

When we assessed the shape of the relation between preeclampsia risk and P*l*GF concentrations we noted the presence of a non-linear (inverted U-shaped) relation between maternal plasma P*l*GF concentrations and the log odds of preeclampsia in this study population (Fig. 1c). Finally, we noted strong positive linear increase in the log odds of preeclampsia risk with increasing sVEGF-R1/P*l*GF ratio up to values of approximately 125; beyond this value, there were too few subjects thus the confidence intervals are wide (Fig. 1d).

Discussion

We found a decreased concentration of VEGF and increased concentration of sVEGF-R1 in the plasma from preeclamptic Sub-Saharan African patients compared with the controls. Maternal plasma P*l*GF concentrations were similar in both cases and controls. These findings agree with some but not all previous reports on these markers and preeclampsia risk.

In maternal blood from Turkish women with preeclampsia, plasma concentrations of VEGF were significantly lower than in normotensive patients (Madazli *et al.* 2003). In a Curacao, Antilles population investigators found decreased serum concentrations of VEGF in preeclamptic women compared to normal controls (Reuvekamp *et al.* 1999). In contrast, Belgian preeclampsia cases had increased concentrations of total VEGF compared with controls (Tsatsaris *et al.* 2003). VEGF concentrations were higher in Egyptian women with preeclampsia as compared with controls (El-Salahy *et al.* 2001). VEGF concentrations in the serum of German preeclamptic women were also significantly higher than in the control group (Bussen *et al.* 2003).

Our results of high concentrations of sVEGF-R1 in plasma of preeclamptic women are consistent with those reported in the literature. sVEGF-R1 concentration in plasma from preeclamptic women was higher than in women with a normal pregnancy (Chaiworapongsa *et al.* 2004, Levine *et al.* 2004). In agreement with this, it has also been shown that placentas from preeclamptic women produce higher concentrations of sVEGF-R1 *in vitro* as compared to controls (Zhou *et al.* 2002, Helske *et al.* 2001). Interestingly, the increase of sVEGF-R1 corresponds to a decrease of free VEGF and P*l*GF in the serum of patients with preeclampsia resulting in endothelial dysfunction (Levine *et al.* 2004). sVEGF-R1 is a major contributor to the pathogenesis of preeclampsia. It has been shown in animal models that the administration of sVEGF-R1 induces hypertension, proteinuria and glomerular endotheliosis in pregnant rats (Maynard *et al.* 2003). However, sVEGF-R1 has been effectively used in other clinical settings. Experimental and clinical administrations of sVEGF-R1 have been successful in the prevention of neovasculogenesis and tumor growth (Yang *et al.* 2001, Chen *et al.* 2000, Stechschulte *et al.* 2001, Goldman *et al.* 1998).

P*l*GF concentration in plasma is significantly attenuated in pregnancies complicated by preeclampsia compared to the controls (Bersinger and Odegard 2004, Reuvekamp *et al.* 1999, Taylor *et al.* 2003, Levine *et al.* 2004). Plasma P*l*GF concentrations in pregnant women rise steadily throughout pregnancy from the levels of non-pregnant women to the levels ten times higher after 30 weeks of gestation (Krauss *et al.* 2004). It has been shown that the additive effect of low concentrations of P*l*GF combined with low concentrations of sex hormone binding globulin may lead to preeclampsia (Thadhani *et al.* 2004).

The quantification of both P*l*GF and sVEGF-R1 has been used for their predictive value in women at high risk of preeclampsia in the first trimester of pregnancy (Thadhani *et al.* 2004). Some investigators have suggested that the ratio sVEGF-R1/P*l*GF may be predictive of preeclampsia risk (Levine *et al.* 2004). In our study, we evaluated preeclampsia risk in relation to the ratio sVEGF-R1/P*l*GF. In agreement with previous reports (Levine *et al.* 2004), preeclamptic patients in our study had a higher ratio sVEGF-R1/P*l*GF compared to controls.

The biological mechanisms for the observed associations of VEGF, sVEGF-R1 and P*l*GF are not completely understood, but it has been suggested the sVEGF-R1 participation as part of one of such mechanisms. Chung *et al.* (2004) found that the mRNA expression of three different VEGF isoforms were

increased, whereas the protein concentrations of two major VEGF isoforms detected were not altered in preeclamptic placentas compared to normal placentas. However, VEGF proteins were elevated in the plasma of preeclamptic patients. The increase of both VEGF protein in plasma and mRNA expression in placenta while the placental VEGF protein expression remains at the same level as in normal placentas is suggestive of a rapid binding of VEGF to sVEGF-R1 and their transport into the circulation. In the same study, sVEGF-R1 protein was found to be increased in preeclamptic placentas compared to normal placentas. Hence, higher concentrations of sVEGF-R1 may contribute to the higher VEGF concentrations in the maternal circulation, while local VEGF protein concentrations in preeclamptic placentas are maintained at the same concentration as normal placentas. This is consistent with the observation that natural and recombinant sVEGF-R1 bind P*l*GF and VEGF with high affinity (Kendall *et al.* 1996), hence natural sVEGF-R1 acting as a VEGF/P*l*GF antagonist *in vivo* (Kendall and Thomas 1993). Further similar differential expression studies should be done on preeclamptic placentas from patients with low VEGF concentrations in the plasma.

The degree and extent of organ damage in disease states may account for the variability of VEGF concentrations in plasma. It has been documented that the source of VEGF, sVEGF-R1 and P*l*GF in preeclamptic women is mainly the placenta (Chung *et al.* 2004). However, VEGF concentrations may be altered secondary to renal dysfunction, alterations of other organs and cell activation (Fava *et al.* 1994, Brown *et al.* 1992a,b, Monacci *et al.* 1993, Roes *et al.* 2004).

Differences in study design, limited statistical power, differences in population characteristics such as maternal age, race/ethnicity, severity of preeclampsia and timing of blood collection are likely to have contributed to the variability of results in various studies. Methodological limitations including failure to adjust for variability in gestational age at blood collection, and technical differences in laboratory analytical procedures and reagents (Levine *et al.* 2004), may all have also contributed to inconsistencies in results from previous studies. Variations in gestational age-specific plasma volume expansion, for instance, have generally not been accounted for in most previous studies. We adjusted for gestational age at blood collection as a means to control maternal plasma volume expansion.

An important limitation must be considered when interpreting our results. Because maternal blood samples were collected when preeclampsia became clinically evident, we cannot determine whether the observed associations between maternal plasma concentrations of the analytes may be attributable to disease-related alterations or whether the alterations in plasma analyte concentrations are casually related to preeclampsia. Findings of alterations of these analytes in plasma collected prior to the clinical diagnosis of preeclampsia (Levine *et al.* 2004) suggest that alterations in maternal plasma VEGF and sVEGF-R1 may be evident as early as at 21 weeks of gestation.

In summary, plasma VEGF, sVEGF-R1 and P*l*GF concentrations are altered among Zimbabwean women with preeclampsia as compared to normotensive women. Prospective studies are needed to evaluate the dynamic changes of these biological markers in maternal and fetal compartments in preeclamptic and normotensive pregnancies.

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