Chronic kidney disease may be differentially diagnosed from preeclampsia by serum biomarkers

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Preeclampsia, affecting 5–8% of pregnancies, is the main cause of fetal–maternal mortality and morbidity. The differential diagnosis with chronic kidney disease (CKD) is a challenge owing to the overlapping clinical features. No biomarker has been found to discriminate between the two conditions. Here, we tested whether maternal serum levels of placental growth factor (PlGF) and soluble FMS-like tyrosine kinase-1 (sFlt-1), markers of preeclampsia, could be used to discriminate between 34 patients with preeclampsia, 23 patients with CKD during pregnancy, and 38 healthy pregnant women. Serum levels of PlGF and sFlt-1 were determined during the third trimester by commercially available immunoassays. In preeclampsia, sFlt-1 levels were significantly increased in comparison with that in CKD and in the control women. Serum levels of PlGF in preeclampsia were significantly decreased relative to both controls and patients with CKD. The sFlt-1 to PlGF ratio was significantly increased in preeclampsia (median 436) compared with controls (median 9.4) and CKD (median 4.0). No differences were found between controls and patients with CKD. Thus, our study suggests that it is possible to discriminate between preeclampsia and CKD during pregnancy by determining maternal serum levels of sFlt-1 and PlGF and their ratio.

Kidney International (2012) **83,** 177–181; doi:10.1038/ki.2012.348; published online 26 September 2012

KEYWORDS: chronic kidney disease; endothelium; glomerulopathy; hypertension

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Received 16 May 2012; revised 6 August 2012; accepted 9 August 2012; published online 26 September 2012

The complex relationship between kidney and placenta has been known since the start of modern medicine. The importance of a differential diagnosis between preexisting kidney disease, eventually exacerbated by pregnancy, and pregnancy complicated by proteinuria and hypertension was underlined over a century ago.[1] Both diseases are frequent and increasing in frequency. Preeclampsia (PE) complicates 5–8% of all pregnancies,[2] whereas chronic kidney disease (CKD) affects 2–3% of women in childbearing age.[1,3,4]

The differential diagnosis between PE and CKD is still a challenge. Both diseases were recently redefined, widening the definitions and pursuing an earlier diagnosis.[3,4] The frequent lack of historical data limits the potentials of a differential diagnosis. Furthermore, glomerulonephritis, as well as systemic immunologic diseases, may appear, relapse, or worsen in pregnancy; some glomerular diseases, such as IgA nephropathy, may cause proteinuria or hypertension in pregnancy.[5–8]

The discrimination between PE and CKD has important consequences. The clinical course of PE is often more aggressive and stormy, and the indications in severe cases are toward early delivery, whereas a wait-and-watch policy may be more rewarding in CKD.[2]

Recent studies have underlined the association between PE and end-stage kidney disease later in life.[4,8] In particular, Vikse *et al.*[4] performed a cohort study on more than one million women with one to three pregnancies and reported that PE could be considered a clinical marker for an increased risk of subsequent end-stage renal disease. This risk is particularly high if PE results in the birth of a low-birth-weight or preterm baby or if PE occurs in more than one pregnancy.[4] The role of undiagnosed CKD in the development of 'early PE' has been a matter of discussion.[4] In spite of the great interest in this complex subject, no clinical criteria or biomarker is currently available to support the differential diagnosis between CKD and PE.

The etiopathogenesis of PE remains elusive. There is evidence indicating that hypertension, proteinuria, and generalized endothelial dysfunction are directly caused by an unbalanced production of placental pro- and anti-angiogenic molecules. In particular, soluble FMS-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) are believed to be pivotal in PE pathophysiology.[9,10] During normal placenta development,

vascular endothelial growth factor and PlGF have a key role in regulating trophoblast growth and differentiation, villous angiogenesis, and remodeling of maternal spiral arteries.[9,10] In PE, the placenta fails to properly invade and remodel maternal uterine spiral arteries, leading to impaired perfusion, hypoxia/ischemia, and oxidative placental stress.[11–13] This condition triggers the overactivation of hypoxia-inducible factor 1α, a main factor in the cellular response to hypoxia.[14] The hypoxia-inducible factor 1α target genes are pro-angiogenic vascular endothelial growth factor and anti-angiogenic sFlt-1, both overexpressed in PE.[15–17] Indeed, abnormally elevated concentrations of placental and circulating sFlt-1, induced by hypoxia/oxidative stress, inhibit free vascular endothelial growth factor and PlGF, thus causing, and/or contributing to, the aberrant placental angiogenesis and generalized endothelial dysfunction.[15,17] Recently, it was demonstrated that vascular endothelial growth factor specifically produced by the podocyte is required to maintain the integrity of the glomerular filtration barrier.[18]

The identification of an sFlt-1/PlGF imbalance as a key event in the pathogenesis of PE has led to new diagnostic and therapeutic perspectives. Both factors are detectable in the maternal circulation at least 5 weeks before the onset of clinical PE, and several groups are working to establish reference values applicable in clinical practice.[17,19,20] In normal pregnancies, maternal serum levels of sFlt-1 continuously increase during pregnancy, whereas PlGF levels increase until the middle of the third trimester and then decrease at the end of pregnancy.[20]

From the pathophysiological point of view, we can expect CKD to behave differently from PE, as CKD patients should have a normal early phase of placental implantation. According to this hypothesis, the sFlt-1/PlGF balance should be normal in pregnant CKD patients in spite of proteinuria or hypertension, thus differentiating between these two conditions.

The aim of this study was to test this hypothesis in a homogeneously followed sample of PE and CKD patients. The results could be of great use in supporting the clinical management of patients with proteinuria and hypertension in pregnancy.

RESULTS

Clinical features of the study population

The study population data are summarized in Table 1. There were no clinically relevant differences in age at the start of pregnancy or prevalence of primiparous mothers. Proteinuria, by definition absent in the controls, was significantly higher in PE than in CKD. The clinical outcomes differed in both groups with respect to controls and were more severe in PE patients (Table 1).

sFlt-1 and PlGF levels and sFlt-1/PlGF ratio in PE, CKD, and controls

Serum levels of sFlt-1 and PlGF, and their ratio, were significantly different between PE and controls and between PE and CKD, whereas no difference was found between CKD and controls (Table 1, Figure 1a). sFlt-1 protein levels were significantly increased in PE relative to both CKD and controls (5.12- and 4.25-fold higher, respectively, $P<0.0001$) (Figure 1a, left panel; Table 1). PlGF serum levels were significantly decreased in PE relative to both CKD and controls (17.4- and 12.5-fold lower, respectively, $P<0.0001$) (Figure 1a, middle panel; Table 1). Receiver operating characteristic curve analysis showed that the sFlt-1 concentration cutoff value to discriminate between PE and CKD patients was 7715 pg/ml (sensitivity 97%, specificity 96%), while for PlGF it was 88.15 pg/ml (sensitivity 90%, specificity 100%).

The sFlt-1/PlGF ratio was significantly increased in PE relative to CKD and controls (about 25-fold higher in both cases, $P<0.0001$), but there was no significant difference between CKD and controls (Figure 1a, right panel; Table 1). Also noteworthy was the lack of overlap of the ranges of the ratio in PE versus CKD and controls (Figure 1b). The sFlt-1/PlGF ratio cutoff value to discriminate between PE and CKD patients was 148.75 (sensitivity 100%, specificity 100%), as determined by receiver operating characteristic curve analysis. Nevertheless, as there is no overlap of the ranges, any value between 136.59 and 160.9 could be equally used to discriminate the two pathologies.

DISCUSSION

PE and CKD have overlapping clinical features, characterized by hypertension and proteinuria, which often render a differential diagnosis impossible. Therefore, the main result of our study was to confirm that PE and CKD differently affect pregnancy and may be differentiated by specific circulating biomarkers (Table 1, Figure 1a and b). We were able to distinguish between PE and CKD by measuring the ratio of circulating sfFlt-1/PlGF levels, whose value seems to discriminate these conditions without any overlap. Therefore, any value of sFlt-1/PlGF ratio between 136.59 and 160.9 could be equally used to discriminate the two pathologies. A cutoff value of 148.75 (rounded to 150) can be suggested.

Thus, our study suggests that the pathogenesis of pregnancy-related problems is different in CKD and PE. In the former, it is linked to the maternal disease, in which the markers of placentation are no different from those of normal pregnancies. In PE, the pathogenesis is linked to a primary defect in placentation. It could be argued that sFlt-1 is a common anti-angiogenic marker, expected to be increased in both pathological conditions. However, we can speculate that in CKD the vascular damage is limited to the kidney, whereas the hypoxic/ischemic PE placenta releases anti-angiogenic and proinflammatory molecules into the maternal circulation, causing generalized endothelial damage.[17]

Recent clinical–experimental studies have related elevated circulating levels of sFlt-1 to anti-neutrophil cytoplasmic antibody–associated vasculitis.[21] In particular, sFlt-1 levels during the acute phase were correlated with the degree of proteinuria.[21] As observed by Stillman and Karumachi,[22]

Table 1 | Clinical characteristics of the study population

	PE (34)[a]	CKD (23)	Controls (38)	*P*-values
Age at start of pregnancy	32.5 ± 4.46	29.5 ± 5.87	31.5 ± 5.37	$P = 0.0327$ $P^1 = NS$ $P^2 = NS$
Primiparous (%)	24/33 (72.7%)	12 (52.2%)	22 (57.9%)	$P = NS$ $P^1 = NS$ $P^2 = NS$
Proteinuria at test (g/day) (Range) (25th–75th Percentiles)	2.62 (0.45–18.36) (1.07–6.60)	0.75 (0.3–6.83) (0.3–1.3)	(Absent)	$P = 0.0002$
Week at test	30.5 ± 3.25	29.8 ± 6.06	34.5 ± 5.12	$P = NS$ $P^1 = 0.0002$ $P^2 = 0.0020$
Week of delivery	30.8 ± 3.15	36.1 ± 2.82	39.1 ± 1.88	$P < 0.0001$ $P^1 < 0.0001$ $P^2 < 0.0001$
Preterm (<37 weeks)	33 (97.1%)	10 (43.5%)	3 (7.9%)	$P < 0.0001$ $P^1 < 0.0001$ $P^2 = 0.0030$
Early preterm (<34 weeks)	27 (79.4%)	4 (17.4%)	1 (2.6%)	$P < 0.0001$ $P^1 < 0.0001$ $P^2 = NS$
Cesarean section (%)	32 (94.1%)	11 (47.8%)	10 (26.3%)	$P = 0.0002$ $P^1 < 0.0001$ $P^2 = NS$
Need for NICU	27/33 (81.8%)	4/22 (18.2%)	3 (7.9%)	$P < 0.0001$ $P^1 < 0.0001$ $P^2 = NS$
Birth weight (g)	1271 ± 531.9	2531.2 ± 633.17	3212.4 ± 486.35	$P < 0.0001$ $P^1 < 0.0001$ $P^2 < 0.0001$
SGA babies <10th percentile (<5th percentile)	13/27-48.1% (3/27–11.1%)[b]	5–21.7% (3–13%)	6–15.8% (3–7.9%)	$P = NS$ $P^1 = 0.0047$ $P^2 = NS$ ($P = NS$ $P^1 = NS$ $P^2 = NS$)
Gestational week at test	30.6 ± 3.25	29.8 ± 6.06	34.5 ± 5.12	$P = NS$ $P^1 = 0.0003$ $P^2 = 0.0020$
sFlt-1 (pg/ml) (Range) (25th–75th percentiles)	13519.5 (6059–34398) (10507–18786)	1988 (692–11172) (1402–2978)	2499 (823–14833) (1737-4070)	$P < 0.0001$ $P^1 < 0.0001$ $P^2 = NS$
PlGF (pg/ml) (Range) (25th–75th Percentiles)	32.6 (11–86.9) (19–44)	426.5 (55.9–2632) (106–626)	279.3 (43.5–1262) (142–578)	$P < 0.0001$ $P^1 < 0.0001$ $P^2 = NS$
sFlt-1/PlGF (Range) (25th–75th Percentiles)	435.79 (160.90–1153.53) (276–703)	4.003 (0.51–136.59) (2.6–20.9)	9.36 (1.38–126.83) (3.1–29.7)	$P < 0.0001$ $P^1 < 0.0001$ $P^2 = NS$

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate; NICU, neonatal intensive care unit; NS, not significant; PE, preeclampsia; PlGF, placental growth factor; sFlt-1, soluble FMS-like tyrosine kinase-1; SGA, small for gestational age.
P = PE versus CKD; P^1 = PE versus controls; P^2 = CKD versus controls.
Only CKD patients who presented with both hypertension and proteinuria were selected for this study. The kidney diseases were as follows: glomerular disease (17.4%), interstitial disease (30.5%), pyelonephritis (13%), polycystic kidney disease (4.3%), kidney transplant (13%), isolated urinary abnormalities (4.3%), and solitary kidney (17.4%). Kidney function was normal (GFR > 90 ml/min) in all controls and PE patients; all but three patients in the CKD group were in stage 1 CKD; three patients were in stage 2 CKD.
[a]One case excluded (intrauterine death).
[b]SGA babies: only cases with gestational age ⩾28 weeks were considered (no reference data below this term).

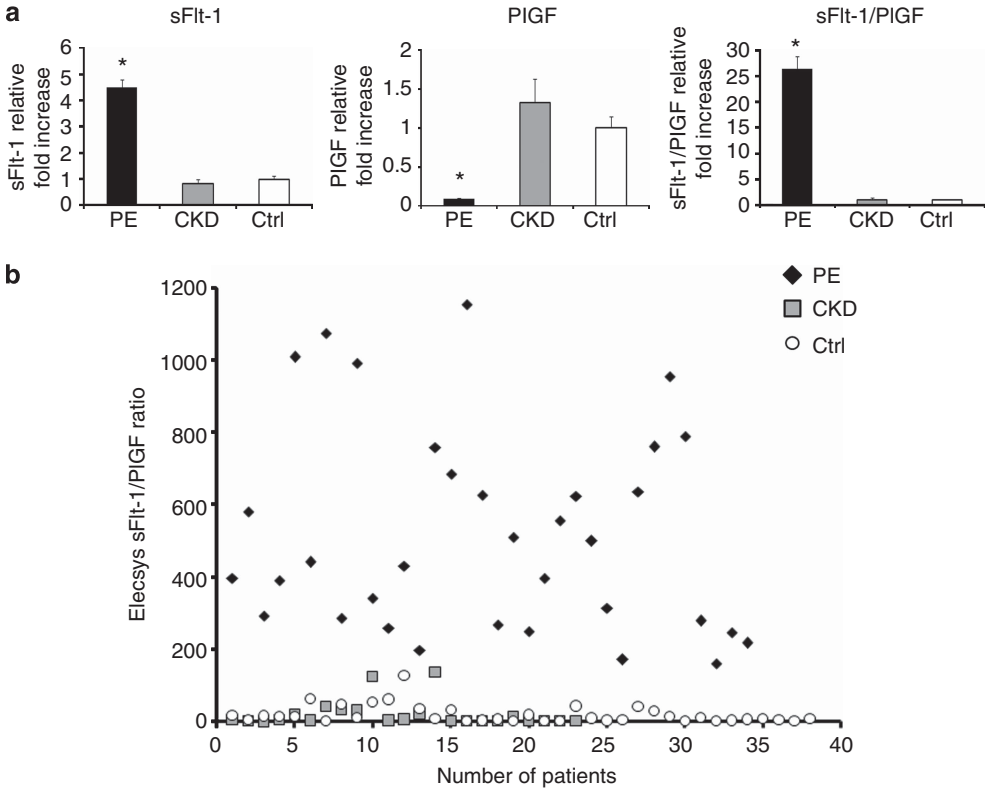

Figure 1 | sFlt-1 and placental growth factor (PlGF) levels and sFlt-1/PlGF ratio in preeclampsia (PE), chronic kidney disease (CKD), and control patients. (**a**) sFlt-1 (left panel), PlGF (middle panel), and sFlt-1/PlGF ratio relative to fold increases in PE versus CKD and control (Ctrl) patients as assessed by the Elecsys methodology. Significance was considered as $P<0.05$. (**b**) Scatterplot of calculated sFlt-1/PlGF ratio. Black squares, PE patients; gray squares, CKD patients; white circles, control patients. *$P<0.001$.

these data emphasize the pivotal role of angiogenic and anti-angiogenic molecules in maintaining blood vessel homeostasis. Indeed, the investigation of circulating markers of disrupted angiogenesis in PE may offer some insights also for diseases characterized by severe endothelial dysfunction, such as vasculitis or severe CKD.

The timely identification of CKD patients should lead to better management of pregnancy, as strict clinical control may allow the fetuses of CKD patients to reach a higher gestational age, whereas in PE a stormy course could more easily be expected and timely delivery is indicated as the only 'cure'.[2,23] After pregnancy, the identification of CKD patients may allow the planning of specific diagnostic and treatment pathways for patients who could otherwise lose this unique occasion for a cure.[23]

The strength of this paper is the proposal of the first biomarkers able to differentiate PE and CKD in pregnancy in a highly significant way, particularly via the sFlt-1/PlGF ratio (Table 1, Figure 1a and b). The main limit of this paper, partly shared by preliminary reports, is the small number of cases enrolled in the study. However, our results for PE and control patients are in line with those obtained in a larger population currently taken as a reference for the commercial kits used.[20] Furthermore, we selected only cases with a full-blown PE picture and with CKD presenting with hypertension and proteinuria; broader recruitment, also including CKD patients without proteinuria and hypertension and patients with pregnancy-induced hypertension, is advisable to provide better insights into these complex and often overlapping conditions. Both limits have been taken into account in the planning of a larger prospective study, to be extended on a multicenter scale, aimed at following larger numbers of both CKD and PE patients and at performing serial tests until delivery and also after delivery.

In conclusion, the results of the present study suggest that sFlt-1, PlGF, and, above all, their ratio are the first biomarkers thus far to differentiate between PE and CKD, a crucial issue to allow the best clinical management of the pregnancies and to allow timely diagnosis of CKD. Larger multicenter studies are needed to best explore the potentials of these tests, which represent the first successful attempt to perform a rapid noninvasive differential diagnosis between these two emerging diseases.

MATERIALS AND METHODS

Study population

The study was conducted at the Maternal and Fetal Medicine Unit of O.I.R.M-Sant'Anna Hospital, University of Turin (Turin, Italy). Patients were recruited and blood samples were obtained after informed consent in accordance with the ethics guidelines of

O.I.R.M.-Sant'Anna Hospital. The study was approved by the Ethics Committee of the Sant'Anna Hospital (no. 335; protocol 11551/c28.2; 4/3/2011).

In total, 34 PE patients, 23 pregnant woman with CKD, and 38 controls (healthy women with uncomplicated pregnancy) were enrolled. PE was defined as hypertension with systolic blood pressure $\geqslant$140 mm Hg or diastolic blood pressure $\geqslant$90 mm Hg accompanied by proteinuria $\geqslant$300 mg/24 h after 20 weeks of gestational age in a previously normotensive woman.[2] CKD was defined as 'every anomaly of blood and urine composition, or imaging or pathological data, lasting for at least 3 months or with a glomerular filtration rate below 60 ml/min for the same time period'.[3,23] Glomerular filtration rate was calculated by the Cockroft–Gault and MDRD formulae on data obtained within 3 months before conception. When this was not available, serum creatinine measured at first control during pregnancy was used.[23] For the sake of this study, only patients with known CKD who presented both hypertension and proteinuria of at least 300 mg/day, i.e., a clinical and laboratory picture superimposable on PE, were selected. Controls had normal pregnancies with no signs of PE, CKD, or other maternal or fetal diseases. Exclusion criteria were twin pregnancies, congenital malformations, chromosomal anomalies, or maternal diseases other than CKD.

The following data were considered for CKD and PE: proteinuria, serum creatinine, glomerular filtration rate, total serum albumin, blood pressure, and antihypertensive therapy, age, parity, race, week of testing, educational level, body mass index, gestational age at delivery, type of delivery, clinical complications in the mother, fetal weight, percentile with respect to the Italian birth weight references, Apgar index, sex, admission to the neonatal intensive care unit, and outcome.

sFlt-1 and PlGF assays

A volume of 2 ml of venous blood was collected from each patient and control using serological vials without anticoagulant and gel. Serum was obtained by centrifugation at 3000 r.p.m. at 4 °C for 20 min within 3 h from collection and stored at -80 °C until testing. All samples were analyzed contemporaneously. Serum levels of sFlt-1 and PlGF were determined in parallel by specific commercially available electrochemoluminescence immunoassays (Elecsys, Roche, Penzberg, Germany) using a Cobas-e-411 immunoanalyzer following the manufacturer's instructions and as previously described by Verlohren *et al.*[20] Normal controls were compared with the reference data supplied by the manufacturer and Verlohren's data.[20] The values of sFlt-1 in the serum of our control pregnancies (3270 ± 405 pg/ml) were comparable to those reported by Verlohren *et al.*[20] (2641 ± 100.5 pg/ml).

Statistical analysis

A descriptive analysis was performed as appropriate (mean and standard deviation for parametric and median and range for nonparametric data). Comparison among groups was performed by analysis of variance. Bonferroni's test was used for *post-hoc* comparisons between two groups of parametric data, while Kruskal–Wallis test was used for nonparametric data and chi-square test for frequencies. Receiver operating characteristic curves were used to determine cutoff values for sFlt-1 and PlGF measurements and their ratio. Significance was set at <0.05.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

The hardware and reagents used in the study were supplied by Roche Pharmaceuticals to TT. The study was presented in abstract form at the SGI (Society for Gynecologic Investigation) Congress, San Diego, 2012.

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