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Original Article

Anatomy Section

Urinary Placental Growth Factor in Pregnancies Complicated by Preeclampsia

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ABSTRACT

Introduction: Preeclampsia is associated with alterations in the maternal serum levels of the vascular endothelial growth factor (VEGF), the placental growth factor (PlGF) and the soluble fms-like tyrosine kinase-1 (sFlt-1). The serial measurement of these angiogenic factors in the serum may be used to pre-empt the diagnosis of preeclampsia, but obtaining such measurements during the routine antenatal care needs a cost-effective analysis. A promising tool is a non-invasive, alternative screening method for measuring the urinary placental growth factor (PlGF).

Objective: To estimate the levels of urinary PlGF in preeclamptic and normotensive, non proteinuric pregnant women in the Indian population and also to analyze the association of this factor with the onset of the disease.

Methods: A case control-study was planned in 80 patients, among which 40 were preeclamptic patients and 40 were normotensive, non proteinuric pregnant women who served as the controls. Urine samples were obtained both from the preeclamptic women and the control women and they were analyzed for the levels of urinary PlGF by using ELISA.

Results: The levels of urinary PlGF were significantly reduced in the preeclamptic patients as compared to those in the normotensive, non-proteinuric pregnant women (30.08 ± 9.42 pg/ml Vs 77.70 ± 24.70 pg/ml, p< 0.0001). Further, the levels were also significantly reduced in early-onset preeclampsia compared to those in late-onset preeclampsia (25.44±6.35 pg/ml Vs 40.92 ±5.71 pg/ml, p<0.0001).

Conclusion: A significant reduction in the levels of urinary PlGF was found in the preeclamptic patients and this reduced level of urinary PlGF may be used to pre-empt the onset of preeclampsia and to institute the appropriate therapeutic measures.

Key Words: Preeclampsia, Placental growth factor, Vascular endothelial growth factor, Vascular endothelial growth factor receptor-1, Soluble fms like tyrosine kinase-1.

INTRODUCTION

Preeclampsia (PE) is a life threatening, pregnancy-specific syndrome which affects 5-10% of the pregnancies and it is characterized by hypertension and proteinuria after twenty weeks of gestation [1,2]. It is a two-stage disorder in which the initial (placental) asymptomatic stage is marked by abnormal placentation [3], followed by the placental elaboration of certain soluble factors which enter into the maternal circulation and cause subsequent widespread endothelial dysfunction, leading to the maternal symptoms of preeclampsia [4]. The placental factors which enter into the maternal circulation include angiogenic factors like the vascular endothelial growth factor (VEGF), the placental growth factor (PlGF) and the soluble vascular endothelial growth factor receptor-1(sVEGFR-1)/soluble fms like tyrosine kinase-1 (sFlt-1).

The serial measurement of the angiogenic factors in the serum might be ideal for ascertaining the risk of preeclampsia [5], but obtaining various serum samples during the routine antenatal care is more labour intensive and invasive as compared to the non-invasive urine samples. Therefore, the estimation of the angiogenic factors by a non-invasive test would be easier and useful in the early assessment of the women who are at a high risk of preeclampsia. One such plausible test may be the estimation of PlGF in urine. Though the levels of VEGF, PlGF and sFlt-1 can be estimated in serum [5], only PlGF acts as a better marker in urine. sFlt1 is a large molecule of 100 kDa and it is usually not filtered into the urine unless there is renal damage [6], whereas urinary VEGF is a small molecule of ~45 kDa and it is present in the cells of the kidney itself (glomerular podocytes and tubular cells). These factors are unlikely to reflect the circulating angiogenic state in the preeclamptic mother [7]. The urinary PlGF is a small molecule of ~30 kDa which is derived from the circulating blood and is filtered by the kidney in the early stages of preeclampsia. Therefore, the estimation of urinary PlGF acts a better and a plausible alternative. Moreover, the incidence of preeclampsia also varies across the world and this variation may influence the levels of the angiogenic factors. Thus, it is important to analyze the baseline value of urinary PlGF in a given population, so as to determine the threshold value for predicting the disease. Hence, the present study was aimed at measuring the levels of PlGF in the urine of preeclamptic and normotensive pregnant women in the Indian population and also in analyzing whether the urinary PlGF was associated with early- and late- onset preeclampsia.

MATERIALS AND METHODS

Study Design

A case-control study was planned with 80 patients, among which 40 women had preeclampsia and 40 women had normal pregnancies. The patients (cases and controls) were selected from the antenatal clinic and the inpatient ward of the Department of Obstetrics and Gynecology, All India Institute of Medical Science, New Delhi, India. The cases and the controls were matched for the maternal age and the gestational age at the time of the urine collection. The preeclamptic women were selected according to the criteria of the International Society for the Study of Hypertension in

Pregnancy (ISSHP) [7]: pregnant women who had systolic and diastolic blood pressures above 140 and 90 mm Hg respectively, in at least two consecutive measurements, at least 4 hours apart, which occurred after the twentieth week of gestation and were accompanied by proteinuria (>300 mg per liter in a 24 h urine collection/>1+ on a urine dipstick). The preeclamptic women were included immediately after the clinical diagnosis, whereas the cases with chorioamnionitis, chronic hypertension, pre-gestational hypertension, renal disease, cardiac disease, active asthma, thyroid disease and epilepsia were excluded from the study. The normotensive, non-proteinuric, pregnant women without any other medical as well as obstetrics complications were enrolled as the control group. The patients with preeclampsia were also sub-classified as either early-onset (< 34 weeks of gestation) or late-onset (>34 weeks of gestation) preeclampsia, according to the gestational age when the preeclampsia had developed. This study was approved by the institutional ethics committee and a written informed consent was obtained from all the women who were enrolled in the study. All the enrolled women were included in the final analysis.

The immuno assay procedure

Urine samples were obtained both from women with preeclampsia (soon after the clinical manifestation of the disease) and age matched (gestational and maternal) normotensive, non-proteinuric, pregnant women who were in 23 to 39 weeks of gestation. The urine samples were collected by the "clean catch" technique. The collected urine samples were then centrifuged at 3000 rpm for 20 minutes, aliquoted and immediately stored at -20ºC. The urinary placental growth factor (PlGF) was measured by a sandwich-type enzyme linked immunosorbent assay (ELISA; Quantikine ® human PlGF, R&D Systems Inc., Minneapolis, MN, U.S.A). According to the kit, the minimum detectable level for PlGF was 9.0 pg/ml and the intra assay variation and inter assay variation were 3.6% and 11.0% respectively.

Statistical Analysis

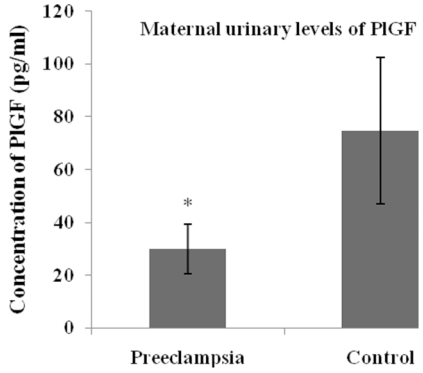
Between the two matched groups, the quantitative variables like systolic blood pressure, diastolic blood pressure, body mass index, urinary protein, uric acid and urinary placental growth factor (PlGF) of the preeclamptic and the control women were compared by using the Wilcoxon sign rank test/paired t- test. The independent t-test was used to compare the urinary PlGF levels between the early-onset and the late-onset preeclampsia cases. The correlation of the urinary PlGF levels with the systolic and the diastolic blood pressure was analyzed by using Pearson's/Spearman's correlation. The result was considered to be statistically significant at a 5% level of significance (i.e., p<0.05). The statistical analysis was performed by using the statistical package, SPSS, version 13.

RESULTS

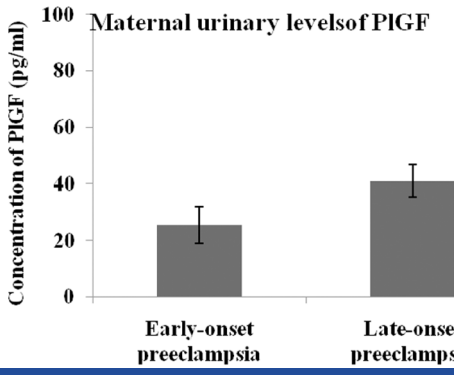
The clinical characteristics of the study population are shown in [Table/Fig-1]. The systolic blood pressure, diastolic blood pressure, body mass index, urinary protein and the uric acid levels were significantly higher in the preeclamptic pregnant women than in the normotensive, non-proteinuric, pregnant women [Table/Fig-1]. The preeclamptic patients had lower levels of the urinary placental growth factor (PlGF) as compared to the women with normal pregnancies (30.08±9.42 pg/ml Vs 77.70±24.70 pg/ml, p< 0.0001) [Table/Fig-2]. When the urinary levels of PlGF in the early-onset preeclamptic group (< 34 weeks of gestation) were compared to those in their matched control group, a significant reduction was observed (25.44 ± 6.35 pg/ml Vs 85.78± 25.87 pg/ml, p<0.0001). A similar pattern was also found in the late-onset preeclamptic group (> 34 weeks of gestation), when the values of the urinary placental growth factor were compared with those in their matched control group (40.92 ± 5.71 pg/ml Vs 48.87 ± 6.14 pg/ml, p<0.05). The urinary levels of PlGF were significantly reduced in the early-onset preeclamptic group (< 34 weeks of gestation) as compared to those in the late-onset preeclamptic group (> 34 weeks of gestation) (25.44± 6.35 pg/ml Vs 40.92 ± 5.71 pg/ml, p<0.0001) [Table/Fig-3]. In contrast, the urinary levels of PlGF were significantly higher in the control women who were at < 34 weeks of gestation than in the control women who were at

	Preeclamptic women (n=40)	Normotensive, non-proteinuric pregnant women (n=40)	Statistical significance (p-value)
Systolic blood pressure (mmHg) Mean+SD	153.8 ± 10.30	113.15 ± 5.43	0.0001**
Diastolic blood pressure (mmHg) Mean+SD	100.75 ± 8.27	75.15 ± 5.08	0.0001**
Body Mass Index Mean+SD	24.91 ± 4.75	22.85 ± 3.50	0.01*
Protein (gm/day) Mean ± SD	5.1± 1.8	0.8 ± 0. 2	0.0001**
Uric acid (mmol/L) Mean ± SD	4.5 ± 1.3	2.7 ± 1.2	0.0001**

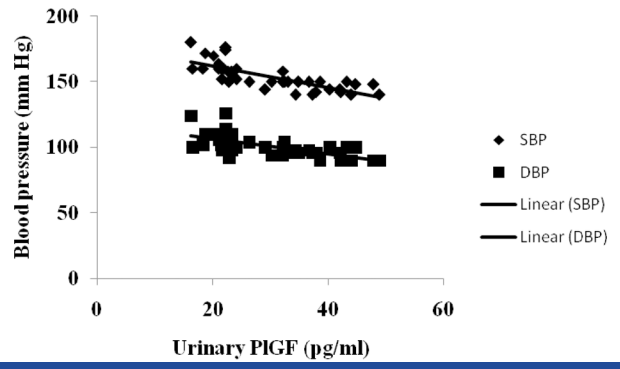
[Table/Fig-1]: Clinical characteristics of pregnant women with preeclampsia and normotensive, non-proteinuricpregnant women at the enrolment of the study



[Table/Fig-2]: Bar diagram represents the urinary levels of Placental Growth Factor (PlGF) in preeclamptic and normotensive, non-proteinuric pregnant (control) women. Values are given as mean+SD. Error bars on the bar diagram represents the standard deviation.* represents the statistical significance, p<0.05.



[Table/Fig-3]: Bar diagram represents the urinary levels of Placental Growth Factor (PlGF) in early-onset preeclampsia and late-onset preeclampsia. Values are given as mean+SD. Error bars on the bar diagram represents the standard deviation. * represents the statistical significance, p<0.05.



[Table/Fig-4]: A scatter plot showing negative correlation of urinary levels of Placental Growth Factor (PlGF) with systolic blood pressure (SBP) and diastolic blood pressure (DBP) in women with preeclampsia.

> 34 weeks of gestation (85.78± 25.87 pg/ml Vs 48.87 ± 6.14 pg/ml, p<0.0001). The correlation of the urinary placental growth factor with the maternal blood pressure was also analyzed in the preeclamptic and the control groups. There was a significant negative correlation between the urinary PlGF and the blood pressure (systolic blood pressure, r = -0.694, p< 0.001 and diastolic blood pressure, r = -0.649, p<0.001) in the preeclamptic patients [Table/Fig-4]. A similar pattern was also found in the control group (systolic blood pressure, r = -0.584, p<0.001 and diastolic blood pressure, r = -0.514, p<0.001).

DISCUSSION

This study found that preeclampsia was associated with the alterations in the levels of the angiogenic factors [8,9] and that these alterations were more pronounced in the early- than in the late-onset preeclampsia cases. In the present study, a significant decrease in the levels of the urinary placental growth factor (PlGF) was found in the preeclamptic women as compared to those in the normotensive, non-proteinuric pregnant women. The reduced levels of urinary PlGF may be due to the reduced circulating PlGF, which in turn was attributed to the increased binding of the free PlGF to the increased circulating soluble fms like tyrosine kinase-1 (sFlt-1) in the circulation [8,10]. sFlt-1 acts as a decoy receptor and binds to the free PlGF in the circulation [11,12], thereby inducing an anti-angiogenic state that may play a causal role in the pathogenesis of the maternal syndrome in preeclampsia. Our previous study also reported higher levels of serum sFlt-1 and lower levels of free PlGF in the sera of preeclamptic women [8].

Although the serum analysis of the angiogenic factors, especially the analysis of serum sFlt-1 could be a better screening method for preeclampsia, obtaining such measurements during the routine antenatal care need a cost-effective analysis. An alternative method is to measure one of these proteins in urine. sFlt-1 is a large molecule of 100 Kda [6] which can't be filtered by the healthy kidney into the urine in the absence of renal damage and VEGF is expressed in podocytes during embryogenesis and in the mature glomerulus. Also, the mRNA for the vascular endothelial growth factor and its receptors, the vascular endothelial growth factor receptor-1 (Flt-1) and the vascular endothelial growth factor receptor-2 (Flk-1) are expressed on the glomerular podocytes, the distal tubules, and the collecting ducts [13-16]. It is therefore unlikely to estimate these factors in the urine of preeclamptic mothers [6]. Urinary VEGF and sFlt-1 acts as poor indicators in reflecting the circulating angiogenic state and thus, urinary PlGF acts as plausible alternative. PlGF is considerably a smaller protein of ~30 KDa and it is not produced by the kidneys. The urinary levels of this angiogenic factor in turn, reflect its circulating levels [17]. Previous studies had reported that the low serum levels of PlGF had antedated the development of preeclampsia [18,19]. Therefore, it is reasonable to expect that the low levels of urinary PlGF would also be a good predictor of preeclampsia. As the variations in the levels of the serum angiogenic factors are reported across the world to be due to geographical, social, economic and racial differences [20-23], we presumed that even the levels of urinary PlGF had variations among the population. Hence, a reference value for the patients of Indian origin would be very beneficial. Thus, in the present study, we estimated the levels of urinary PlGF in the preeclamptic patients of the Indian population.

In order to analyze whether urinary PlGF was associated with the onset of the disease, we estimated the levels of urinary PlGF in the early- and late onset preeclampsia cases. A significant reduction in the levels of urinary PlGF was found in the early-onset (< 34 weeks of gestation) as compared to the late-onset preeclampsia (>34 weeks of gestation) cases. In contrast, the level of urinary PlGF was much higher in the control women at < 34 weeks of gestation as compared to that in the control women at >34 weeks of gestation. This was consistent with the findings of previous longitudinal studies, where increasing levels of PlGF had been found during the normal pregnancy upto the end of the second trimester, followed by a decrease during the third trimester [6]. We also analyzed the association of urinary PlGF with the maternal syndrome. A significant negative correlation was found between the urinary PlGF and the systolic and the diastolic blood pressure in preeclamptic women. This indicated that low levels of urinary PlGF were also associated with the severity of the disease.

Since preeclampsia contributes to significant maternal morbidity and mortality in India, a prompt diagnosis and intervention are of vital importance in its management. The burden of preeclampsia on the health care resources may be improved by reducing the delay in the patient's decision to seek care, the delay in the patients' arrival at the hospital or the tertiary care centre and the delay in the provision of adequate care, especially the delay in the diagnosis of the disease. The delay in the diagnosis can be considerably mitigated by a reliable, valid and an economical test that could predict pre-eclampsia. One such test may be the urinary test for PlGF. A noninvasive, urine test would be superior to one that requires a blood draw. A urinary test for PlGF could probably be performed less expensively than could a blood test for sFlt-1, because it wouldn't require the services of a medical professional to draw blood. Moreover, a urine sample could conceivably be collected at home, and then brought into a medical lab for testing. This would be an advantage over a blood test, especially in the countries which lacked trained medical staff to draw the blood. Once the preeclamptic women were identified through this urine PlGF test, studies could be targeted to find effective ways to prevent its progression or to keep the most dangerous complications from occurring.

CONCLUSION

Our study suggested that preeclampsia could be associated with low levels of urinary PlGF at the time of the clinical manifestation. Further analysis will be required to clarify the regulation and the secretion of these angiogenic factors in women with preeclamp-

sia. A rapid, non-invasive screening of the preeclamptic women, based on the angiogenic factors, may be used to define the severity of the disease and this appeared to be superior to the routine urinary protein measurements. A prospective study on the levels of urinary PlGF before and after the onset of preeclampsia is progressing in our lab. A cost effective analysis which is done by the serial measurement of urinary PlGF during the routine antenatal care can not only pre-empt the diagnosis of preeclampsia, but it can also bring about an improvised therapeutic management and decrease the stagnating MMR (maternal mortality ratio); thereby improving the maternal health in India.

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