Research Article

Serum Levels of Soluble Fms like Tyrosine Kinase-1(sFlt-1) in Normotensive and Preeclamptic Pregnancy

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Abstract

Background: The study aimed to measure serum levels of sFlt-1 throughout pregnancy in normotensive and preeclamptic women and to determine if sFlt-1 concentrations alter before the clinical manifestation of the condition. **Material and Methods:** It was a nested case control study. Serum samples were collected during pregnancy at three different gestational periods (12-20 weeks, 21-28 weeks and 29- till term) and one sample post delivery, from within the cohort of pregnant women reporting to antenatal clinic. The subjects were divided in to normotensive (controls) and preeclamptic (cases) groups. Circulating levels of antiangiogenic (sFlt-1) were analyzed by enzyme linked immunosorbent assay (ELISA). **Results:** Maternal serum concentrations of sFlt-1 increased with the advancement of gestation age in both normotensive and preeclamptic pregnancies but significantly more increased levels were observed in preeclamptic pregnancies as compared with normotensive pregnancies. **Conclusion:** Maternal circulating sFlt-1 concentrations were significantly higher in women with preeclampsia, which may contribute to the development of preeclampsia.

Keywords: Pregnancy, Preeclampsia, Soluble Fms like Tyrosine Kinase-1.

Introduction

Preeclampsia, a life-threatening pregnancy specific syndrome, characterized by pregnancy induced hypertension (BP ≥140/90 mm Hg) and proteinuria of 300 mg/24 hour or more after 20 weeks of gestation[1]. Preeclampsia is a medical disorder during pregnancy that could lead to high risk of fetal growth retardation, premature delivery and even maternal death[2, 3]. Alternative splicing of the VEGF receptor Flt-1 results in the production of an endogenously secreted antiangiogenic protein referred to as sFlt1, which lacks the transmembrane and cytoplasmic domain of the membrane-bound receptor [4]. Thus sFlt1 can antagonize vascular endothelial growth factor (VEGF) and placental growth factor (PLGF) by binding to them and preventing interaction with their endogenous full-length receptors[5]. There is substantial evidence that increased production of sFlt-1 plays a major pathogenic role in the severe

endothelial dysfunction of preeclampsia. However, more recent studies have shown that circulating sFlt-1 is increased in the amniotic fluid in preeclampsia and in serum at the onset of preeclampsia in late gestation[6, 7].

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Although the primary trigger for abnormal placental development and excess sFlt1 production in preeclampsia remains speculative, our work suggests that excess sFlt1 may be sufficient to produce generalized endothelial dysfunction and some of the clinical phenotype noted in preeclampsia.The present study therefore was undertaken to estimate the maternal serum sFlt-1 concentrations throughout pregnancy among the women who subsequently developed preeclampsia, compared with their concentrations in normotensive pregnant women.

Materials and Methods

Study design & setting: This was a tertiary care teaching hospital based nested case control study. **Participants**: With the approval of the institutional ethics committee and a written informed consent from each woman, a total of 105 healthy women with singleton pregnancy of 12-20 weeks of gestation were included in this study.

The gestational age of women, at the time of collection was determined by ultrasonographic examination. The women with history of essential hypertension, renal disease, epilepsy, diabetes or any other chronic or preexisting disease were excluded from the study. Peripheral blood samples were collected from recruited subjects at four time periods; first at 12-20 weeks (weeks), second at 21-28 weeks and third sample at 29 weeks of gestation to till term. The fourth blood sample i.e. the end point samples were obtained at 48 hours post delivery. Serum was separated and stored in multiple aliquots at -80^{0}c for estimation of sFlt-1. The sample collections were done from all women blinded to their preeclamptic or normotensive status. Out of 105 women registered, only 15 women developed preeclampsia. The criteria for diagnosis of preeclampsia were elevation of blood pressure ≥140/90 mm Hg (measured at least 24 hours apart) and proteinuria of ≥300 mg/24 hour or more after 20 weeks gestation.

Since the number of cases was significantly less than the number of controls therefore for each woman with preeclampsia, one normotensive control was selected from the cohort and matched according to gestational age.

Estimation of sFlt1 by serum ELISA:

Estimation of sFlt1 was determined by a commercially available enzyme linked immunosorbent assay (ELISA) kit, as per manufacturer's instructions, for sFlt1 (e-Bioscience, USA). Samples were run in duplicate. The minimal detection limit of assay for sFlt1 was <30 pg/ml.

Statistical analysis

Data were summarized as Mean ± SE. Groups were compared by repeated measures two factor (Periods x Groups) analysis of variance (ANOVA) using general linear models (GLM) and the significance of mean difference within and between the groups was done by Newman-Keuls post hoc test. A two-sided (α=2) $p<0.05$ was considered statistically significant.

Results

Table 1: Demographic and clinical characteristics (mean±SE) of two groups

Characteristic	Controls (n=15)	Cases (n=15)	p value
Age (year)	25.66±1.01	25.80±1.07	0.929
Weight (kg)	54.46±2.40	54.93±1.85	0.879
Height (cm)	148.46±1.23	146.46±1.04	0.226
BMI (kg/m^2)	24.75±1.16	25.65±0.90	0.54
SBP (mm Hg) at 1st visit	116.93±1.20	118.93±1.82	0.369
DBP (mm Hg) at 1st visit	69.33±0.74	70.26±0.64	0.352
GA at delivery (weeks)	39.00±0.25	36.80±0.35	0.000
Infant's birth weight (kg)	3.01±0.08	2.66±0.12	0.028

The demographic and clinical characteristics of two groups are summarized in Table 1. At presentation, the demographic characteristics viz. age, weight, height, BMI, SBP and DBP were similar ($p>0.05$) between the two groups i.e. not differed statistically.

However, clinical characteristics gestational age (GA) at delivery lowered significantly ($p<0.001$) in cases as compared to controls. Further, infant's birth weight was also significantly ($p<0.001$) lower in cases as compared to controls.

Table 2: sFlt1 levels (Mean ± SE, n=15) of two groups during gestational age

Groups	Gestational age			
	12-20 weeks	**21-28 weeks**	**29-till term**	**Post delivery**
Control	281.67 ± 22.77	417.00 ± 44.39^{a}	1372.33 ± 93.80^{ab}	274.73 ± 30.86^{bc}
Cases	^{ns}298.47 ± 33.15	^{ns}511.46 ± 61.03^{a}	***6929.73 ± 1005.27^{ab}	^{ns}324.20± 45.32^{bc}

Between groups:

$^{ns}p>0.05$ or $^{*}p<0.05$ or $^{**}p<0.01$ or $^{***}p<0.001$- Control vs. Cases

Within groups:

$^{a}p<0.05$ or $^{a}p<0.01$ or $^{a}p<0.001$- as compared to 12-20 weeks

$^{b}p<0.05$ or $^{b}p<0.01$ or $^{b}p<0.001$- as compared to 21-28 weeks

$^{c}p<0.05$ or $^{c}p<0.01$ or $^{c}p<0.001$- as compared to 29-till term

The sFlt-1 levels of two groups over the periods are summarized in Table 2 also shown graphically in Fig. 1. The mean sFlt-1 levels in both groups increases up to 29- till term and thereafter decreases comparatively at post delivery.

Further, at all periods, the level of it was higher in cases than controls. Comparing the mean sFlt-1 levels within the groups (i.e. between

periods), the sFlt-1 levels in both groups were significantly ($p<0.001$) higher at 21-28 weeks and 29- till term as compared to 12-20 weeks.

Further, in both groups, the level of it was also significantly ($p<0.001$) higher at 29-term as compared to 21-28 weeks. However, in both the groups, it lowered at post delivery as compared to other periods.

Similarly, comparing the mean sFlt-1 levels between the groups, the sFlt-1 levels differed significantly ($p<0.001$) at 29 till term, but did not differed significantly ($p>0.05$) at 12-20 weeks and 21-28 weeks between the two groups.

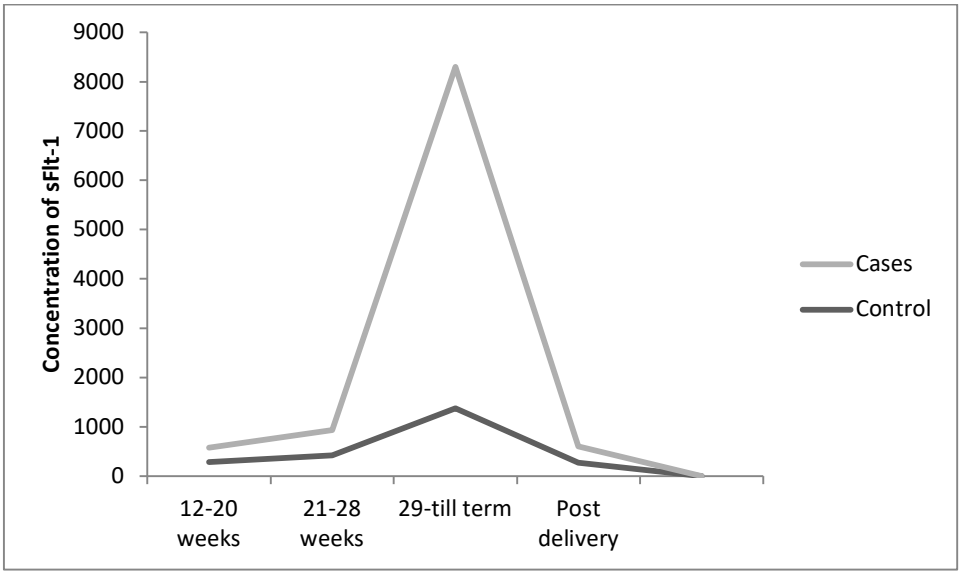

Fig 1: The sFlt-1 levels of two groups over the periods (Pregnancy up to delivery)

Discussion

Role of imbalance of angiogenic factors in the genesis of preeclampsia has been proposed by several investigators[8, 9, 10, 11]. Maynard et al. (2003), in their experimental model have shown development of preeclampsia like phenotype in pregnant rats, when induced with high circulating sFlt, suggesting that the antiangiogenic factor sFlt might have a pathogenic role in preeclampsia[9].

In the present study we evaluated the levels of sFlt-1 with the progression of gestational age in both normotensive and preeclamptic women. We observed that the levels of sFlt1 were increased in both normotensive (controls) and preeclamptic women (cases) with the progression of gestational age and decreased thereafter in later gestation. But in women who developed preeclampsia sFlt1 levels increased more up to 29 weeks and later on showed a declining trend in these women as compared to controls.

Studies of sFlt-1 have shown conflicting results. Levine et al[8] found elevated levels of circulating sFlt-1 five weeks before clinical preeclampsia, but detected no association with sFlt-1 in second trimester. Two other studies by Thandani et al[12] and Power et al[12] failed to show any association with sFlt-1 but Hertig et al showed that high concentration of sFlt-1 late in second trimester was associated with increased risk of preeclampsia.[13]

Our study showed rapid fall in sFlt1 levels in post partum period in both controls and cases. Similar observations have been made by other workers[12]. The decrement in our study however was lesser in preeclamptic group. Possible explanation for this

slower decrease could be due to either slower excretion or higher rate of production as placenta is not the only site for production of sFlt[14] significantly disturbed normal mechanism of sFlt1 clearance from the body in preeclamptic women cannot be ruled out[13].

Our study also had the same limitation. We did not look for the alterations in the angiogenic markers in other obstetric conditions similar to preeclampsia, such as gestational hypertension and pregnancy induced hypertension(PIH). Since preeclampsia is a syndrome so no single test will predict preeclampsia risk, for better results combination of marker required in our study we did not look for the other angiogenic markers levels like VEGF and PLGF.

We were able to analyze samples collected prior to the onset of symptoms in all preeclamptic cases from a large

prospective, carefully monitored cohort. Thus, we were able to minimize selection bias.

The study needs to done on a large sample before making a final statement on its significant impact reliable prediction and management of preeclampsia. It would allow earlier diagnosis of preeclampsia and closer prenatal monitoring.

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Conflict of interest: Nil

Permission from IRB: Yes

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