Original Article

Pattern of sFlt-1 Antiangiogenic Serum Marker in Pregnancy-Induced Hypertension in Sharif Medical City Hospital, Lahore

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ABSTRACT

Objective: To investigate the maternal serum level of antiangiogenic factor sFlt-1 in pregnancy-induced hypertension (PIH) at different gestational ages in Sharif Medical City Hospital (SMCH), Lahore.

Methodology: The present descriptive case-control study evaluated maternal serum levels of antiangiogenic marker sFlt-1 in 90 women with gestational age 18-40 weeks referred to SMCH, Lahore. It included 69 pregnant women with hypertension and 21 normotensive pregnant control subjects. The hypertensive patients were taking methyldopa. Out of 69 hypertensive pregnant subjects, 24 had gestational hypertension (GH), 41 had preeclampsia (PE) and 4 had eclampsia (EC). The indicators of blood pressure, urinary albumin and convulsions were the principals in categorizing the hypertensive pregnancy in GH, PE and EC. The age, parity, BMI, urinary albumin and hemoglobin were also noted and measured. The antiangiogenic serum biomarker was analyzed and assessed with enzyme-linked immunosorbent assay (ELISA).

Results: The average serum concentration of sFlt-1 was 33% lower in hypertensive pregnant women as compared to normotensive control subjects. The difference was statistically significant (p=0.05). In GH, sFlt-1 concentration was 28% lower than controls. In PE, sFlt-1 level was 37% lower than the normotensive subjects and in EC patients, sFlt-1 level was 25% lower than the normotensive subjects. It had been observed that PE subjects exhibited a maximum decrease in sFlt-1 among all hypertensive subjects. There is also a significant difference in sFlt-1 levels at different gestational ages.

Conclusion: The serum sFlt-1 levels in pregnancy-induced hypertension women are higher than normotensive women. In our study, the serum levels of antiangiogenic factor sFlt-1 in pregnancy-induced hypertension are lower than normotensive pregnant women. This is attributed to the use of methyldopa in these patients.

Keywords: *Antiangiogenic marker sFlt-1. Pregnancy-induced hypertension. Preeclampsia. Eclampsia.*

INTRODUCTION

Hypertension in pregnancy is common worldwide and accounts for 12% maternal mortality during pregnancy and puerperium.[1] Hypertensive disorders in pregnancy (HDP) cause severe maternal obstetric complications, preterm delivery, fetal intrauterine growth restriction, low birth weight and perinatal death. The different categories of HDP are gestational hypertension, preeclampsia and eclampsia.

The probable causes of pregnancy induced hypertension are abnormal placentation, vasculopathy, inflammatory changes, immunological factors, genetic factors and nutritional factors.[2] The incidence of PIH varies with age, parity, geographic distribution and socioeconomic status. The risk factors for pregnancy-induced hypertension are age under 20 and over 35 years, first pregnancy, previous history of severe PIH, family history of preeclampsia, short stature, migraine, chronic renal disease and diabetes mellitus.[3]

Although the etiology remains unidentified, placental hypoperfusion and diffuse endothelial cell injury are considered to be the central pathologic events. Reduced perfusion as a consequence of abnormal placentation is supposed to lead to ischemia-reperfusion damage to the placenta.[4] In pregnancy-induced hypertension, there is an insufficient maternal vascular response to placentation leading to ischemic changes. Various noxious substances are released from the placenta and deciduas. These serve as mediators to provoke endothelial injury.[4,5]

Recent data demonstrates that antiangiogenic proteins including soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) and proangiogenic protein placental growth factor (PLGF) are expressed in large amounts various weeks prior the onset of clinical indications and symptoms of PIH.[5] An imbalance of placental-derived antiangiogenic factors may play a main role in facilitating endothelial dysfunction. Alternative splicing of Flt-1 results in the formation of sFlt-1. This cannot fasten to cell membranes and is secreted into the maternal bloodstream leading to high serum levels. It can antagonize vascular endothelial growth factor (VEGF) and placental growth factor by binding to them and avoiding their interface with endogenous receptors.[5]

Khalil et al. suggested that alpha methyldopa may have

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Received: August 1, 2017; Accepted: August 30, 2017.

a precise effect on placental and/or endothelial cell function in preeclampsia mothers, changing angiogenic and antiangiogenic proteins. Methyldopa has been correlated with a noteworthy reduction in maternal serum of sFlt-1.[6,12,13]

The incidence of pregnancy-induced hypertension is comparatively higher in Pakistan. Thus to understand the pathogenesis, sFlt-1 is assessed in different categories of HDP to understand its association with the disease. It may assist in prediction and diagnosis of pregnancy-induced hypertension.

METHODOLOGY

This case-control descriptive study was performed over the period of six months. Written informed consent was obtained from all patients participating in the study and they were assured about the privacy of data. The study was approved by ethics committee of the institution.

Ninety pregnant women with gestational age between 16 weeks to term (38 weeks), referred to SMCH were enrolled in the study. Sixty nine women were hypertensive and 21 were normotensive. The hypertensive patients were taking methyldopa. After obtaining informed consent, 3 ml blood sample was taken using aseptic measures. The relevant history was filled in questionnaires including age, obstetric history, smoking habits, headache, blurred vision and medication intake. Gestational age was based on last menstrual period and first trimester or early second-trimester ultrasound.

Pregnant women with the blood pressure more than 140/90 mmHg in the twentieth week of gestation on two occasions at least six hours apart were labeled as hypertensive. The hypertensive patients without proteinuria were included in the category of gestational hypertension (GH), with proteinuria more than 300mg/24 hrs or 1+ protein on the dipstick with midstream urine sample were categorized in preeclampsia, convulsions with or without proteinuria were grouped as eclampsia. The inclusion criteria for all groups were age 18 and 40 years, singleton pregnancy, non-molar gestation, nonsmoker and no history of hypertension before pregnancy. The exclusion criteria were patients with the history of chronic hypertension, renal diseases, liver disease, cardiovascular disease, diabetes and other problems that may threat mother or fetus. Out of 69 hypertensive pregnant women, 24 had gestational hypertension, 41 had preeclampsia and 4 had eclampsia. The serum level of sFlt-1 was determined by ELISA kit.

STATISTICAL ANALYSIS

In the comparison of various groups, the mean & standard error of mean was calculated and the significance of difference between groups and subgroups was determined with an independent t-test. The data were analyzed using SPSS version 21.0. The significance of difference was taken at $p < 0.05$.

RESULTS

The average age in normotensive and hypertensive pregnant subjects was 18-30 years. Sixty nine hypertensive and 21 normotensive pregnant women were included. In this study, most of the hypertensive pregnant women were of younger age. Preeclampsia affected subjects had increased body mass index (BMI). Hypertension was common in multiparous subjects compared to nulliparous women. Out of 69 hypertensive patients, 24 patients were categorized in gestational hypertension, 41 in preeclampsia and 4 in eclampsia.

The average concentration of sFlt-1 in normotensive control pregnant subjects was 3956±681ng/L. The mean value of sFlt-1 in hypertensive subjects was found 2619±234.5ng/L. In hypertensive subjects, sFlt-1 level was 33% lower than normal subjects and the difference was statically significant (p=0.05). There had been noticeable differences in sFlt-1 levels in different categories of hypertensive subjects. In GH, sFlt-1 concentration was 2825±395ng/L which was found to be 28% lower than controls. In PE, sFlt-1 level was 37% lower than the normotensive subjects and in EC patients, sFlt-1 level was 25% lower than the normotensive subjects. It had been observed that PE subjects exhibited maximum decrease in sFlt-1 among all hypertensive subjects. In PIH and EC, the difference was not significant (Table 2 and Fig. 1).

During 18-28 weeks of pregnancy, there was 19% increase in the marker concentration in GH whereas it exhibited lower concentration in other categories of hypertension compared to the controls. There was however no significant difference among all hypertensive groups during 18-28 weeks of gestation in comparison to normotensive group.

Table 1: Distribution of the study subjects in different categories of hypertension

Distribution	Total	Normotensive	Hypertensive	GH	PE	EC
No. of Subjects	90	21	69	24	41	4
Percentage	100	23.33	76.67	26.67	45.5	4.5

Table 2: sFlt-1ng/L in the study subjects

Groups	Number	Mean±SEM	Difference of hypertensive patients to controls (%)	p-value
Normotensive	21	3956±681.0		
Hypertensive	69	2618±234.5	33% lower	0.05
GH	24	2824±395.3	28% lower	0.160
PE	41	2466±312.5	37% lower	0.005
EC	4	2944.87±88.65	25% lower	0.540

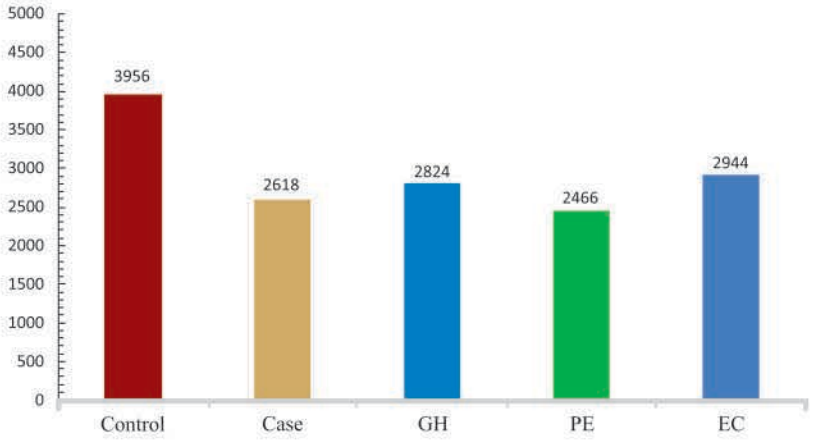

Figure 1: Comparison of sFlt-1ng/L in the study subjects

During 29-35 weeks of gestation, the marker concentration was lower in all hypertensive cases except EC. The PE group was the only one which was statistically significant (p = 0.068).There was 37% decrease in the marker concentration in PE whereas it exhibited 8% higher concentration in EC group compared to the controls. There was however no significant difference in PIH during this phase of gestation in comparison to normotensive (control) group. During 36-40 weeks of gestation, sFlt-1 levels are decreased in all hypertensive patients but it is non-significant statistically.

DISCUSSION

In normotensive pregnancy, antiangiogenic factor soluble fms like tyrosine kinase receptor 1(sFlt-1) level is constant during the first and middle trimester of gestation and there is a marked increase beginning at 33-36 weeks. According to different studies, there is a marked variation in serum marker levels within the same gestational age. It may be due to the difference in sample handling, processing and laboratory procedures. There is no definitive cut-off value to predict pregnancy induced hypertension.[14] So far numerous studies provided the association between the alteration of angiogenic and antiangiogenic factors and subsequent development of pregnancy induced hypertension.[7] Similar findings were reported in other studies.[8,9]

In our study, sFlt-1 level was 33% lower than the normotensive pregnant normal subjects and the difference was statically significant (p=0.05). Additionally, there had been noticeable differences in sFlt-1 levels in different categories of hypertensive subjects. In GH, sFlt-1 concentration was found to be 28% lower than controls. In PE, sFlt-1 concentration was 37% lower and in EC, it was 25% lower than the pregnant normotensive subjects. It had been observed that PE subjects exhibited the lowest decrease in sFlt-1(37%) among all the hypertensive subjects. In PIH and EC, the difference was not significant. (p=0.16, p=0.54).

In our study, the analysis of this factor concentrations in different phases of pregnancy had been performed; the phases were 18-28, 29-35 and 36-40 weeks of the pregnancies. The concentrations of sFlt-1 were markedly greater in GH and comparatively lower in PE and EC collectively from the normotensive pregnancies at 18-28 weeks. The level of the factor was lowered at 29-35 weeks of pregnancies in GH and PE than the normotensive subjects. There was 8% increase in sFlt-1 in EC group. At 36-40 weeks, sFlt-1 levels are decreased in all hypertensive patients but it is non-significant statistically. The pattern is certainly revealing a relationship between the factors and the phases of pregnancies in our sample population.

In PIH cases, there is amplified antiangiogenic property thus causing inconsistent angiogenesis. A patterned appearance of the angiogenesis-related factors is the requirement during pregnancy and changes in the pattern is the reason or result of the vascular disorder.[8,10,11] The hypertensive study subjects were taking methyldopa. There are a few reports regarding the influence of methyldopa on the angiogenesis related humoral factors. Khalil et al. showed that antihypertensive drugs like methyldopa may have a definite role in placental and endothelial cell function in pregnancy-associated hypertension patients, leading to decreased antiangiogenic sFlt-1 in preeclampsia but not in gestational hypertension.[6]

In our study, it has been observed that administration of methyldopa (Aldomet) significantly decreased the level of serum sFlt-1 levels with a positive effect on the control of the disease. The present study, however, observed there was reduced serum level of sFlt-1 at different gestational ages of hypertensive pregnant women as compared to normotensive control pregnant women at the same gestational ages. This contrast could be due to discrepancies in the study population (for example age, parity, BMI, ethnicity, smoking position, genetics) or on the definite onset of HDP versus the diagnosis of HDP.

CONCLUSION

The serum sFlt-1 levels in PIH women are higher than normotensive women. In our study, the serum levels of antiangiogenic factor sFlt-1 in pregnancy-induced hypertension are lower than normotensive pregnant women. This is attributed to the use of methyldopa in these patients.

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