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ORIGINAL ARTICLE

Pattern of Pro-Angiogenic Serum Placental Growth Factor in Gestational Hypertensive Women

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ABSTRACT

Objective: The aim of this study was to investigate maternal serum levels of an angiogenic factor called Placental Growth Factor (PLGF) at different ages of pregnancy in gestational hypertensive women residing in Raiwind Lahore, Pakistan.

Methods: The analytical cross-sectional study was conducted at the Department of Obstetrics and Gynecology Sharif Medical City Hospital, Lahore from January 2015 - April 2015. Serum levels of PLGF were measured and compared between 22 control normotensive subjects (Group A) and 18 gestational hypertensive (Group B). Moreover, comparison was also made among different gestational weeks groups (I, II and III).

Results: Serum PLGF levels were raised in gestational hypertensive (Group B) compare to normotensive (Group A) [366 (185-695) vs. 156 (132-319) ng/L], (P= 0.024). Serum PLGF levels were 156 (132-319) in gestational hypertensive individuals and 366 (185-695) ng/L in normotensive individuals. Serum levels PLGF of 18 -28 (I), 29-35 gestational weeks (II) were raised [425 (197-452) vs. 163 (91-332)], [546 (309-1519) vs. 137 (86-147)]. While in last 36-40 weeks group (III) levels were decreased [216 (136-881) vs. 315 (185-612)] in comparison of normotensive subjects.

Conclusion: It is concluded from this research that placental–derived angiogenic biomarker PLGF plays a significant role in gestational hypertension. The pattern of the PLGF marker in various intervals of pregnancy may provide information for the management of hypertension in pregnancy.

Keywords: PLGF, Gestational hypertension, Angiogenesis, Placentation, Methyledopa

INTRODUCTION

Pregnancy induced hypertension (PIH) is defined as a rise in blood pressure as a consequence of pregnancy. There are three categories of PIH, gestational hypertension (GH), preeclampsia and eclampsia.[1] The overall percentage of maternal mortality due to hypertension is high at 12% and has a very extensive impact on the nation's healthcare system.[2] It complicates about 6-10% of pregnancies.[3] and accounting for about a quarter of all anti natal admissions in UK.[4] Rehman *et al.*(2003) assessed the incidence of PIH among gravid woman population in Karachi, (37%) women were identified to be the cases of GH; 23% of the cases were found to be of old age; 72% of the cases were primigravida and rest were multigravida. Results show a good prevalence of PIH cases among gravid women in Karachi.[5]

Hypertensive disorder of pregnancy causes severe maternal obstetric complications, including fetal intrauterine growth restriction, low birth weight, preterm delivery and perinatal death. The probable causes of pregnancy induced hypertension are abnormal placentation, vasculopathy, inflammatory changes, immunological factors, genetic factors and nutritional factors.[6] The risk factors for pregnancy induced hypertension are age under 20 and over 35 years, first pregnancy, and previous history of pregnancy induced hypertension, family history of preeclampsia, short stature, migraine, diabetes mellitus and chronic renal diseases.[7] Gestational hypertension is defined as a blood pressure measurement of 140/90mmHg for the first time after 20 weeks of pregnancy without detectable proteinuria.[8]

Although the etiology remains unidentified, placental hypoperfusion and diffuse endothelial cell injury are considered to be the central pathological events. Reduced perfusion as a consequence of abnormal

placentation is supposed to lead to ischemia reperfusion damage to the placenta.[9]

Placental angiogenesis is a fundamental process that establishes feto-maternal circulation, ensures well-organized maternofetal exchanges, acting as central mechanistic role in the expansion of the placental villous tree, and contributes to the overall progress of the placenta throughout pregnancy. Failure in these processes is tightly linked to the development of placental pathologies such as pregnancy induced hypertension, early pregnancy loss, and intrauterine growth restriction (IUGR).

Placental growth factor (PLGF) is member of vascular endothelial growth factor (VEGF) family that is made predominantly in placenta. PLGF is focal in vasculogenesis and regulation of micro vascular permeability. The placenta (oxidatively stressed) releases anti-angiogenic proteins such as soluble fms-like tyrosine kinase-1 (sFlt-1), prostaglandins and cytokines into the maternal circulation. Meanwhile, the hypoxic placenta decreases the production of pro-angiogenic factors including placental growth factor (PLGF) and vascular endothelial growth factor (VEGF). These variations cause the systemic endothelial dysfunction. An inflammatory reaction that leads to raised systemic vascular resistance, vasoconstriction, stimulation of the coagulation cascade. In the end clinical manifestations such as hypertension, proteinuria, neurological, hematological disturbances, hepatic dysfunction, and fetal growth restriction are observed. The incidence of pregnancy induced hypertension as a result of hypoxic placenta is relatively high (37%) in pregnant women of Pakistan.[10]

Increased occurrence of hypertension syndrome in pregnancy determines the necessity to find out the threat factors which assist to detect a group of patients with high risk of serious complications onset. In addition, even mildly increased BP in pregnancy was shown to be related with an increased risk of developing complications, including those associated to placentation process abnormality.

Thus, to understand the pathogenesis and its association with the disease we assessed PLGF in gestational hypertensive and normotensive subjects. It may assist in predication and diagnosis of hypertensive disorder in pregnancy.

METHODS

The analytical cross-sectional study was conducted in the Department of Obstetrics and Gynecology Sharif Medical City Hospital, Lahore, three months duration January 2015 - April 2015 after taking permission from the Head of Department and Ethical review board. Forty gravid women with gestational age between 18 to 40 weeks were enrolled in this study. 22 were normotensive control subjects and 18 were hypertensive without proteinuria labeled with gestational hypertension. The inclusion criteria for all groups were age ranging between 18-40 years, singleton pregnancy, non-molar, nonsmoker and no history of hypertension before pregnancy. Patients with the history of chronic hypertension, renal, liver, cardiovascular diseases, diabetes and other problems that may threat mother or fetus were excluded from the study. A comparison of maternal serum level of PLGF in gestational hypertensive and normotensive subjects was conducted in forty subjects.

The venous blood sample of 3cc was taken using aseptic measures. The blood sample was immediately transferred to serum vacutainer. The blood was kept in vials for about 20-25 minutes to clot. It was centrifuged at 5000RMP for 10 minutes. The clear serum was pipette out into 2ml vial (Appendrof, Germany). The samples were stored in freezer at -80 degree centigrade. PLGF levels were estimated in serum by ELISA using Human PLGF kit (Glory science co.,Ltd USA, Catalog #12831,Product code#23026362, 74,000. PKR).

From outdoor patients department (OPD) patients fulfilling inclusion criteria were included in this study after taking a written informed consent. The relevant history was filled in questionnaires including age, obstetric history, smoking habits, headaches blurred vision and medication intake. Gestational age was based on last menstrual period and first trimester or early second trimester ultrasound.

Data were analyzed using SPSS-21. Data for serum PLGF were described as median (IQR). Serum PLGF levels were compared in hypertensive subjects by using Mann-Whitney U test. The significance of difference was taken at $p \leq 0.05$.

RESULTS

There were total forty pregnant women, 18(45%) were hypertensive and 22 (55%) were normotensive included in this study. Serum PLGF levels were raised in gestational hypertensive (Group B) compare to normotensive (Group A) [366 (185-695) vs. 156 (132-319) ng/L], (P= 0.024) Table.1.

Serum levels of PLGF were raised in 18-28 weeks (group I) and 29-35 weeks (group II) while decreased in 36-40 weeks (group III) of gestation of hypertensive subjects as compare to normotensive. There was statistically significant difference in group II levels of PLGF. (p=0.003). (Table 2)

Table 1. Comparison of serum PLGF (ng/L) in the Group A (Normotensive control) and Group B (GH)

	n	Median (IQR)	p-value*
Groups			
Group A (Normotensive control)	22	156 (132-319	0.024
Group B (GH)	18	366 (185-695))	

GH: Gestational hypertensive, IQR: Interquartile Range, PLGF: Placental Growth Factor
*p< 0.05 significantly different statistically

Table 2: Comparison of PLGF ng/L levels between Normotensive (control) and Gestational hypertension (GH) in different gestational weeks groups (I, II and III)

Groups (Gestational weeks)	Subjects	n	Serum PLGF ng/L	p-value*
			Median (IQR)	
Group I (18-28 with Methyledopa)	GH	7	425 (197-452)	0.053
	Control	7	163 (91-332)	
Group II (29-35 with Methyledopa)	GH	5	546 (309-1519)	0.003
	Control	8	137 (86-147)	
Group II (36-40 without Methyledopa)	GH	6	216 (136-881)	0.295
	Control	7	315 (185-612)	

GH: Gestational hypertensive, PLGF: Placental Growth Factor, IQR: Interquartile Range
*p< 0.05 significantly different statistically

DISCUSSION

Pregnancy accompanies appearance of numerous hormones and humoral factors for adaptations of developing fetus in the maternal environment. Vascularity is the principal system in these adaptations and the process of angiogenesis is essential for the survival of fetus in fetal maternal association. It is significantly influenced by various humoral factors, angiogenic and non-angiogenic factors. The disorders related to vascularity as hypertension in pregnancies have been found to be associated with changes in angiogenic and anti-angiogenic factors circulatory concentration. Normal placental development is possible only under normal instruction of angiogenic processes; on the other hand, hypertensive developments are frequently associated with vascular complaints.[11] The role of placenta in hypertensive state of pregnancy is described by different previous studies.[12,13]

A patterned appearance of the angiogenesis related factors is the requirement during pregnancy and changes in the pattern is the reason or result of vascular disorder. It is imagined that, in GH cases there is amplified anti-angiogenic property thus causing inconsistent angiogenesis, resulting in diminished villouscapillarization.[10,13] Whereas there are reports of no difference in villous capillarization of normotensive and GH subjects.[14] Some studies observed lower PlGF levels in gestational hypertensive (GH) mothers in contrast to normotensive during antepartum and intrapartum phases.[15] The angiogenic factor PLGF has been concerned in the mechanism and foretelling value.[16,17] Few other studies demonstrated comparison of angiogenic biomarkers patterns in high risk pregnancies and low risk pregnancies. Abnormal placental production of angiogenic factors, consist of sFlt-1 and PLGF contribute to endothelial dysfunction in pregnancy induced hypertensive states by an antagonizing endothelial protective vascular endothelial growth factor (PLGF and TGF-beta) in maternal circulation.[18-20]

PLGF levels in women who later had preeclampsia were substantial lower than the controls from 13-16 weeks of gestation till delivery and the PLGF was lowest in women with clinical preeclampsia at similar gestational age group.[21,22]

In our study the primary objective was to observe the pattern of specific placental pro-angiogenic serum marker PLGF for gestational hypertension (A & B group) and in different phases of pregnancy (I, II and III groups). The concentrations of PlGF in the different

groups demonstrated a pattern. In groups I and II PLGF levels were greater while in group III later stage of pregnancies it decreased, lower than normotensive pregnancies. This pattern is certainly revealing a relationship between the factor (PLGF) and the phases of pregnancies in our sample population; the mechanisms in these patterns involved require further researches on the aspects to understand adequately.

As there are a few reports regarding the influence of methyl DOPA on the angiogenesis related humoral factors showed that antihypertensive drugs like methyldopa may have definite role in placental and endothelial cell function in pregnancy associated hypertension patients. Further pro-angiogenic factor i.e. PLGF was not affected by administration of methyldopa. Methyldopa treatment had no important effect on the serum levels of PLGF in women presenting with gestational hypertension.[23]

But in our study, it is contradicted to previous study that administration of Alpha-Methyldopa in group I & group II had significant role in women with GH, by increasing indirectly pro angiogenic effect by increasing PLGF serum levels. This finding is further strengthened by decreasing of PLGF in group III following discontinuation of alpha metyledopa after thirty-five weeks of gestation. This result shows the significance of circulating pro-angiogenic factor PLGF in the pathophysiology of GH, as less is identified on how this biomarker fluctuates over the duration of pregnancy and after delivery. In 2015 Shah & Khalil conducted a study on the "bioactive factors in uteroplacental and systemic circulation link placental ischemia to generalized vascularized dysfunction in hypertensive pregnancy and preeclampsia" which report the imbalance in the levels of placental growth factor in preeclampsia as compared to normal pregnancy.[24]

As there is no ultimate cut-off value to predict to Hypertensive disorder of pregnancy (HDP)[22]Extremely inadequate biomarker screening, follow up studies and increased occurrence of hypertension syndrome in pregnancy determines the necessity to find out the threat factors which assist to detect a group of patients with high risk of serious complications onset. In addition, even mildly increased BP in pregnancy was shown to be related with an increased risk of developing complications including those associated with placentation process abnormality.

The results of present study strongly recommended that further investigations on these aspects on large scale in the population keeping in view lifestyle and especially genetic in all the categories of pregnancy associated hypertension. This may reveal either additional placental production of PLGF or reduced binding to local circulating and membrane-bound receptors, but the particular mechanisms for this need further investigations. A contradicted relationship between PLGF and GH observed in our study is representative of findings from locally assorted group of gravid women. It is enlightening that results from our study present a conflicting data compared to some former cross sectional studies mentioned.

CONCLUSION

The serum levels of pro angiogenic factor PLGF are decreased in GH women. In our study, the serum levels of Pro angieogenic factor PLGF are raised in group I and group II, who were on Methyledopa while decreased in group III patients, following discontinuation of Alpha methyledopa. Our findings suggest that Alpha methyl DOPA has an indirect pro angiogenic effect by increasing levels of PLGF in Gestational Hypertensive women. However, more randomized studies should be conducted so that specific and inclusive results regarding serum PLGF in gestational hypertension can be achieved.

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ETHICAL APPROVAL: This study was approved by Institutional Review Board of the University of Lahore, Pakistan.

CONFLICT OF INTEREST: All authors do not have any conflict of interest.

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