Original Article

Levels of Placental Growth Factor (PLGF) in Preeclamptic Gravid Women Taking Methyldopa

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ABSTRACT

Objective: To estimate the serum levels of pro-angiogenic placental growth factor (PLGF) in preeclamptic gravid women taking methyldopa at different gestational ages in Sharif Medical City Hospital (SMCH), Lahore.

Methodology: This cross-sectional comparative study was performed over a period of six months after approval by the ethical committee of the institution. A total of 62 gravid women with gestational age between 18 weeks to term (40 weeks) presenting to the Obstetrics & Gynaecology Department of Sharif Medical City Hospital (SMCH) were enrolled in the study. They were categorized into 41 women with preeclampsia and 21 gravid normotensive subjects (as a control group) by stratified sampling technique. After obtaining informed written consent, a 3 ml blood sample was taken using aseptic measures and serum PLGF levels were estimated using human soluble PLGF enzyme-linked immunosorbent assay (ELISA) kit. The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 24.

Results: The average serum concentration of PLGF was higher (536±77 ng/L) in preeclamptic gravid women than normotensive gravid women (206±29 ng/L). A statistically significant difference was observed in both groups (p=0.004). The levels of PLGF were increased in gravid women with preeclampsia during 18-28 and 29-35 weeks of pregnancy whereas the PLGF level was decreased during 36-40 weeks in preeclamptic women.

Conclusion: It has been concluded that the serum levels of pro-angiogenic factor PLGF were higher in gravid women with preeclampsia taking methyldopa than normotensive pregnant women. This is credited to the use of alpha methyldopa in these patients. This study suggests that alpha methyldopa has a precise positive effect in increasing PLGF levels and improving placental & endothelial cell function in preeclamptic gravid women.

Keywords: *Pro-angiogenic factor. Placental growth factor. PLGF. Preeclampsia. Alpha methyldopa.*

INTRODUCTION

Preeclampsia (PE) is characterized by hypertension and proteinuria in gravid mother and can lead to growth restraint of fetus developing at late pregnancy. Hypertensive diseases of pregnancy such as persistent hypertension, pregnancy-induced hypertension (PIH) and preeclampsia remain principal reasons for maternal and neonatal morbidity and mortality. Preeclampsia occurs in 5-8% pregnancies all over the world.[1]

According to the International Society for the Study of Hypertension in Pregnancy (ISSHP) classification, preeclampsia is defined as newly onset hypertension of more than 140/90 mmHg after 20 weeks gestation, proteinuria greater than 300 mg/day or a spot urine protein/creatinine ratio ≥30 mg protein/mmol creatinine.[2]

In preeclampsia, the reendothelialization of cytotrophoblasts is diminished and the route of spiral arteries into myometrium is insufficient, resulting in small-caliber resistance vessels. Placental ischemia and hypoxia occur due to depressed placentation. The initiating event in preeclampsia has been assumed to be reduced uteroplacental perfusion as a consequence of atypical cytotrophoblast invasion of spiral arterioles.[3,4]

The molecular mechanisms of preeclampsia depend upon angiogenic factors which also contribute to its chief phenotypes such as hypertension and proteinuria. Nowadays novel anti-angiogenic proteins including soluble endoglin (sEng), soluble FMS-like tyrosine kinase-1 (sFlt-1) and one pro-angiogenic protein i.e. placental growth factor (PLGF) are expressed in varying levels at different gestational ages. An imbalance of pro and antiangiogenic serum biomarkers formed by the placenta play a role in endothelial dysfunction.[4,5]

Pro-angiogenic placental growth factor is the main participant of the vascular endothelial growth factor (VEGF) family and it is formed principally by the placenta. It has a key role in angiogenesis and trophoblastic invasion of the maternal spiral arteries. Maternal serum levels of PLGF are decreased in 11 to 13 weeks of gestation leading to impaired placentation and preeclampsia.[5]

The etiology of PE is associated with dysregulation in angiogenic factors.[6] It has been reported by different studies that the plasma/serum PLGF concentration in women with preeclampsia is lower than in controls with normal blood pressure. The reduction in serum PLGF concentration is observed at the commencement of early second trimester (after 12th week of gestation) of

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hypertensive gravid subjects.[7,8]

Alpha methyldopa is one of the drugs used for the treatment of hypertension and the management of PE in gravid women. Methyldopa stimulates central alpha-adrenergic receptors by alpha-methyl norepinephrine resulting in a decreased sympathetic outflow of norepinephrine to the heart, kidney and peripheral vasculature.[9]

Alpha methyldopa may have a precise effect on endothelial cell function and/or placenta in PE mothers by changing angiogenic proteins. It is not yet clear whether angiogenic factors are concerned with the pathophysiology of PE directly or are merely indicators of the course of illness however antihypertensive treatment with alpha methyldopa has been correlated with a noteworthy reduction in mothers serum levels of sFlt-1 (anti-angiogenic) and upsurge in maternal serum of PLGF (pro-angiogenic) which plays a supportive role in the switch of progression of illness.[9]

Imbalance of pro and anti-angiogenic factors produced by the placenta may perform the main part in facilitating dysfunction of endothelium which leads to the progression of hypertensive disease of pregnancy. This study was conducted to determine the levels of pro-angiogenic factor PLGF in women with preeclampsia taking methyldopa and compare them with control subjects. It will help to understand the pathogenesis of preeclampsia and its management with alpha methyldopa. It will also have an impact on decreasing morbidity and mortality in these patients.

METHODOLOGY

This cross-sectional comparative study was performed over a period of six months after approval by the ethical committee of the institution. A total of 62 gravid women with gestational age between 18 weeks to term (40 weeks) presenting to the Obstetrics & Gynaecology Department of Sharif Medical City Hospital (SMCH) were enrolled in the study. They were categorized into 41 women with preeclampsia and 21 gravid normotensive subjects (as a control group) by stratified sampling technique. The inclusion criteria for the PE group was age 18-30 years, singleton non-molar pregnancy, nonsmoker, no history of hypertension before pregnancy and taking alpha methyldopa. The exclusion criteria for both groups were patients with a history of persistent hypertension, kidney disease, liver disease, cardiovascular illness and diabetes mellitus that may risk the mother or fetus and any metabolic disorder before or after pregnancy. The diagnosed hypertensive gravid women with BP ≥ 130/90 mmHg and proteinuria greater than 300 mg/24 hrs or 1+ and more on the dipstick in the 20th week of gestation with midstream urine sample were labeled as having preeclampsia. The relevant history was filled in questionnaires including age, presenting complaints, obstetric history, smoking habits and medication intake. The duration of gestation was calculated by the last menstrual period (LMP), further confirmed by first or early second trimester ultrasound. After obtaining informed written consent, a 3 ml blood sample was taken using aseptic measures and serum PLGF levels were estimated using human soluble PLGF enzyme-linked immunosorbent assay (ELISA) kit. The estimation of PLGF was done in different periods of pregnancy i.e. 18-28, 29-35, and 36-40 weeks of gestation.

STATISTICAL ANALYSIS

The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 24. In the comparison of various groups, the mean and standard error of the mean was calculated. An independent t-test was applied to compare the levels of pro-angiogenic PLGF serum marker in preeclamptic and normotensive subjects. The significance of difference was taken as p-value ≤0.05.

RESULTS

This study included 41 women with preeclampsia and 21 normotensive pregnant control subjects. The subjects with PE had an increased body mass index (BMI). The mean level of PLGF in preeclamptic pregnant women was 536±77 ng/L whereas the average concentration of PLGF in normotensive control subjects was 206±29 ng/L. The PLGF levels were significantly higher in preeclamptic gravid women than normotensive control subjects (p-value =0.004). These results are shown in table 1 & figure 1.

During 18-28 weeks of pregnancy, there were increased levels of PLGF factor in preeclamptic gravid women (524±149.6 ng/L) as compared to normotensive gravid women (198±49.41ng/L). The difference was significant statistically (p=0.05) (Table 1 & Figure 2).

During 29-35 weeks of pregnancy, the mean level of PLGF in gravid women with preeclampsia was 615±116.55 ng/L whereas the mean level of PLGF in normotensive control subjects was 193±46.24 ng/L. The results were not statistically significant in this gestational period (p=0.056). These results are shown in table 1 and figure 3.

In 36-40 weeks of pregnancy, the mean levels of PLGF in preeclamptic and normotensive gravid women were 189±39 ng/L and 311±82 ng/L, respectively. There was no statistically significant difference with a p-value=0.174 (Table 1 & Figure 4).

DISCUSSION

Preeclampsia is a hypertensive disorder of pregnancy beginning from the 20th week of gestation and is characterized by proteinuria, hypertension and multiorgan dysfunction. Hypertension in pregnancy is

Table 1: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) at Different Gestational Age

Gestational Age (Weeks)	Groups	Number of subjects (n)	Mean±SEM	p-value
18-40	Preeclamptic Gravid Women (Taking Methyldopa)	41	536±77	0.004
	Normotensive Gravid Women (Control Group)	21	206±29	
18-28	Preeclamptic Gravid Women (Taking Methyldopa)	14	524±149.6	0.056
	Normotensive Gravid Women (Control Group)	7	198±49.41	
29-35	Preeclamptic Gravid Women (Taking Methyldopa)	17	615±116.55	0.155
	Normotensive Gravid Women (Control Group)	9	193±46.24	
36-40	Preeclamptic Gravid Women (Taking Methyldopa)	10	189±39	0.174
	Normotensive Gravid Women (Control Group)	5	311±82	

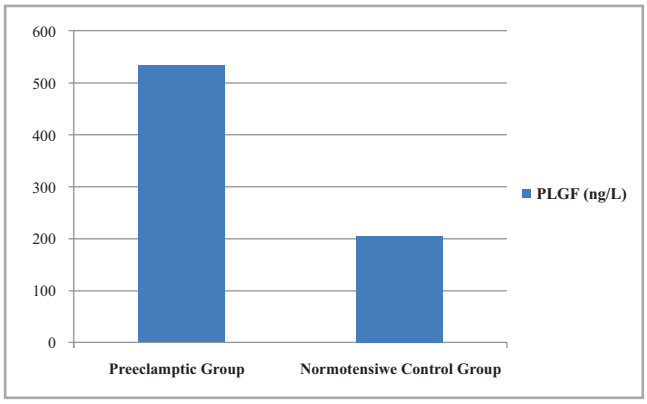

Figure 1: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) during 18-40 Weeks

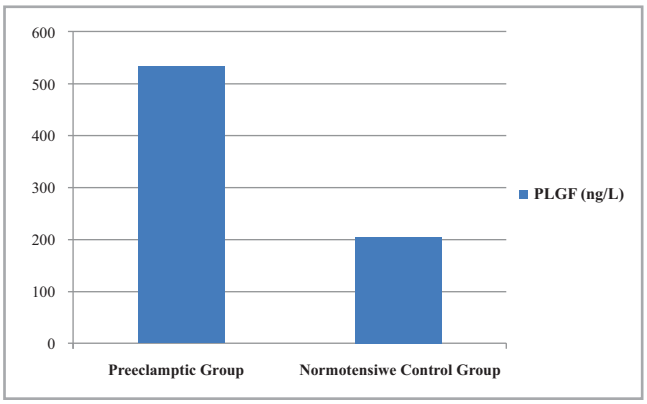

Figure 2: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) during 18-28 Weeks

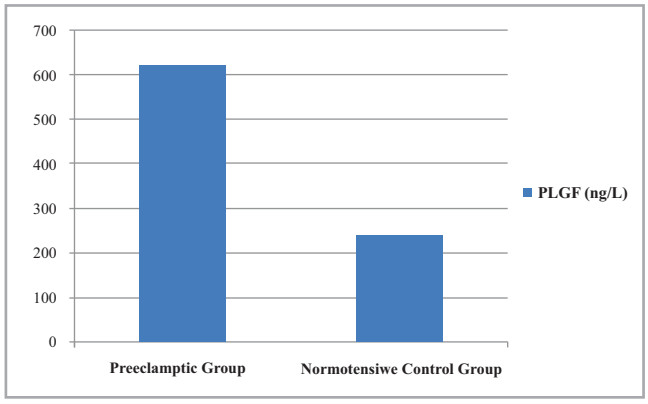

Figure 3: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) during 29-35 Weeks

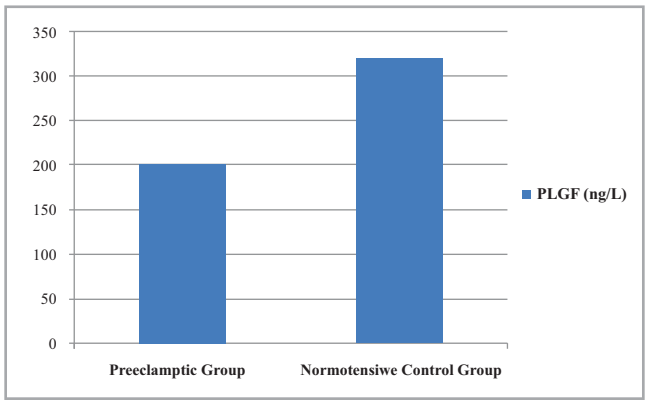

Figure 4: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) during 36-40 Weeks

associated with high morbidity and mortality.[10] In Pakistan, the incidence of pregnancy-induced hypertension is comparatively high.[11] However, biomarker screening and follow up studies are extremely insufficient.

Pregnancy accompanies the appearance of numerous hormones and humoral factors as a part of the adaptations of developing a fetus in the maternal environment. The process of angiogenesis is crucial for the survival of a fetus in the fetal-maternal association. Hypertension in pregnancy has been found to be correlated with changes in the circulatory level of angiogenic and anti-angiogenic factor and vascular complaints.[12,13]

In normotensive pregnancies, pro-angiogenic factor PLGF levels differ during different gestational ages. According to different studies, there is marked variation in PLGF levels within the same gestational

age. It may be due to the difference in sample handling, processing and laboratory techniques. There is no definite cutoff value to predict PE and other hypertensive disorders of pregnancies.[14] Some studies provided the association between the alteration of angiogenic proteins (sFlt-1 & PLGF) and subsequent development of preeclampsia. Hirashima et al. reported that the concentration of PLGF is decreased before the onset of hypertension in pregnancy particularly in PE.[15] Maynard et al. and Levine et al. both observed a decline in PLGF levels in women with preeclampsia taking no antihypertensive medicines.[16,17] But in our study, PLGF levels were higher in preeclamptic pregnant women than control subjects with normal blood pressure and the difference was statistically significant (p=0.004). The PE gravid women were on methyldopa which may have a definite role in improving placental & endothelial cell function and increasing the levels of pro-angiogenic proteins. The analysis of this factor (PLGF) concentrations in different periods of pregnancy was performed and the periods were18-28, 29-35, and 36-40 weeks of gestation. The serum levels of PLGF were high (524±14 ng/L) in 18-28 weeks with statistically significant difference (p=0.056), highest (615±11 ng/L, p=0.15) in 29-35 weeks but declined (189±39 ng/L, p=0.174) near the term in 36-40 weeks, indicating that there is no definite cutoff value of PLGF levels in PE gravid women. But the pattern is surely revealing a relationship between the PLGF factor and PE in different phases of pregnancy.

There are a few studies reporting the influence of methyldopa on the angiogenic factors. According to a study by Khalil et al., the antihypertensive drugs like methyldopa have no protective effect on PE gravid women by changing the proangiogenic (PLGF) proteins in pregnancy.[9] This is in contrast to the present study which indicates a significant alteration in PLGF angiogenic protein levels in PE gravid women of 18-40 weeks (p=0.004).

The present study observed that there were amplified maternal serum levels of PLGF in PE gravid women at 18-28 weeks and 29-35 weeks of pregnancy as compared to the normotensive control group. In 36-40 weeks of pregnancy, serum levels of PLGF were decreased in preeclamptic gravid women than normotensive subjects.

The increasing level of serum PLGF may reveal either additional placental production of PLGF or reduced binding to local circulating and membrane-bound receptors, but the particular mechanism for this needs further investigation. The results of the present study strongly recommend further research on the effect of alpha methyldopa on the levels of serum proangiogenic factor PLGF in preeclamptic gravid women on a large scale.

CONCLUSION

It has been concluded that the serum levels of pro-angiogenic factor PLGF were higher in gravid women with preeclampsia taking methyldopa than normotensive pregnant women. This is credited to the use of alpha methyldopa in these patients. This study suggests that alpha methyldopa has a precise positive effect in increasing PLGF levels and improving placental & endothelial cell function in preeclamptic gravid women.

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