Original Article

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Assessment of Maternal Serum Levels of Vascular Endothelial Growth Factor and Placental Growth Factor in Threatened Abortion: A Case Control Study

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF) are implicated in the pathophysiology of Threatened Abortion (TA). VEGF family regulates placental angiogenesis and maternal spiral artery remodelling whereas PlGF is an important regulator of decidual angiogenesis and is a mediator of trophoblast function. Earlier studies have investigated role of these markers in patients with TA but no definite consensus has been reached. Moreover there is limited data from our population.

Aim: To assess the maternal serum levels of VEGF and PlGF in patients with TA compared to uncomplicated pregnancy.

Materials and Methods: This was a case control study conducted in a tertiary care hospital of Northern India in which total 40 TA patients and 40 controls were analysed. A 3 mL of peripheral blood sample was collected from each case and control. The plasma was separated and quantification of VEGF and PlGF was done by using ELISA kits in cases and in age and gestational age matched controls.

Results: Serum VEGF level was significantly lower in cases compared to controls (30.65±9.41 pg/mL vs. 64.55±16.67 pg/mL, p<0.001) irrespective of their gestational age. TA patients also had lower serum PlGF level (263.54±68.108 pg/mL vs. 341.83±112.704 pg/mL, p<0.001). But no definite trend was found in VEGF and PlGF levels with increasing gestational age.

Conclusion: Maternal serum VEGF and PlGF level was low in patients with TA compared to uncomplicated pregnant women regardless of gestational age.

Keywords: Angiogenic factors, Placenta, Spontaneous abortion, Syncytiotrophoblast

INTRODUCTION

TA is one of the most common complication of pregnancy and is reported in about 20% of normal pregnancy and 15-20% of them end up in spontaneous abortion [1]. It is defined as vaginal bleeding with or without pelvic pain upto 20 weeks of gestation when the cervical os is closed and there is cardiac activity on ultrasonography [2].

TA is generally not associated with serious maternal morbidity and mortality but the risk for adverse pregnancy outcomes such as preterm birth, premature rupture of membranes, low birth weight, foetal growth restriction, placental abruption and caesarean delivery increases [2]. Unlike other types of abortion, the pathophysiological mechanisms resulting in TA are not fully understood [1]. Vaginal bleeding during early pregnancy most commonly originates from placenta and is an outcome of defective placentation, which results in altered synthesis and release of angiogenic and anti-angiogenic factors by placenta in the maternal circulation such as VEGF and PlGF [3,4]. Another hypothesis suggests impairment of placento-decidual interface in early pregnancy leads to premature and excessive entry of maternal blood inside the placenta. This causes loss of syncytiotrophoblast function and lower levels of VEGF and PlGF [5-7]. VEGF family is known to regulate placental angiogenesis and maternal spiral artery remodelling whereas PlGF is an important regulator of decidual angiogenesis and mediator of trophoblast function [8,9].

Since long, studies have postulated the role of VEGF and PlGF in pathogenesis of TA and few studies have also suggested their role as biomarkers in these patients [1,4,5,10]. But the values of VEGF and PlGF in TA varied widely in different studies, making their interpretation difficult at this point of time. Moreover, there is scarce Indian data evaluating these angiogenic factors in patients of TA. Thus, the present study was conducted to assess if there was any significant difference of maternal VEGF and PlGF levels in patients of TA compared to uncomplicated pregnant women in a cohort of North Indian patients.

MATERIALS AND METHODS

This was a case control study conducted in the Department of Obstetrics and gynaecology in collaboration with the Department of Biochemistry at University College of Medical Sciences and Guru Teg Bahadur Hospital Delhi,India from November 2015 to April 2017. Ethical clearance was taken from the institutional ethical committee.

Sample Size Calculation

In the earlier study by Keskin U et al., the median VEGF level in cases of TA was 39.10 ng/mL compared to 5.24 ng/mL in uncomplicated pregnancy [1]; with a difference of 34 ng/mL. So to estimate a difference of 34 units at alpha=5% and power=80% a sample of 15 cases was required. Whereas, the PlGF value was 20±10 ng/mL in TA versus 30±20 ng/mL in controls. Considering this a sample size of 40 cases was calculated for the present study to have a power of 80% and alpha error of 5%.

So for the present study 40 TA cases were enrolled and divided into 3 gestational age groups of 6-10, 11-15 and 16-20 weeks of gestation [Table/Fig-1]. Considering adverse pregnancy outcomes like spontaneous abortion, missed abortion or any other complication and drop out rates, for every case age matched and gestational age group matched controls were enrolled and followed-up till 20 weeks of gestation. Pregnant women in the control group who developed adverse pregnancy outcomes were excluded from the final analysis. At the end of the study we analysed the serum levels of VEGF and PlGF in 40 cases and 40 age and gestational age group matched controls. The same study population was used for another publication by same authors [11].

Gestational age groups in weeks	Cases (n=40)	Controls (n=40)
6-10	13 (32.5%)	13 (32.5%)
11-15	20 (50%)	21 (52.5%)
16-20	7 (17.5%)	6 (15%)

[Table/Fig-1]: Distribution according to gestational age.

Inclusion Criteria for Cases

Primigravida or multigravida pregnant women with bleeding per vaginum with or without pain in abdomen from 6-20 weeks of gestation in age group between 18-35 years with ultrasound confirmed intrauterine pregnancy with or without choriodecidual haemorrhage, internal os closed and normal cervical length upto 2.5 cm were taken.

Exclusion Criteria for Cases

Pregnant women of more than 20 weeks of gestation with history of previous spontaneous abortion or any previous pregnancy complication such as pregnancy induced hypertension, gestational diabetes mellitus, intrauterine growth restriction, intrauterine death, preterm labour, premature rupture of membranes or any medical disorder such as diabetes mellitus, hypertension, asthma, antiphospholipid antibody syndrome, thyroid dysfunction, tuberculosis and other chronic disorder were excluded from cases. Any women with history of diagnosed uterine anomaly and cervical insufficiency or any uterine surgeries like myomectomy, septoplasty, adhesiolysis for Asherman's syndrome except previous caesarean section were also excluded.

Inclusion Criteria for Controls

Asymptomatic pregnant women in age group of 18-35 years upto 6-20 weeks period of gestation with an uncomplicated pregnancy at time of enrolment were taken and exclusion criteria for controls was same as that of cases.

An informed consent was taken and a detailed general physical and local gynaecological examination on each subject was carried out followed by ultrasonography.

A 3 mL of peripheral blood sample was collected from each case and control in EDTA vial. The plasma was separated by centrifuging at 2000 rpm for 10 minutes and stored at -80° Celsius till further analysis. Quantification of VEGF (Krishgen biosystems, Mfd-10/2017, REF KB1155) and PlGF (Krishgen biosystems, Mfd-10/2017, REF PLGF1016) was done by using commercially available ELISA kits.

STATISTICAL ANALYSIS

Microsoft Excel (version 2007) and statistical software SPSS for Windows (version 20.0) was used for data presentation and statistical analysis. Chi-square test was used to compare categorial variables. Repeated measure ANOVA test was applied followed by Tukey's test for comparison of VEGF and PlGF in cases and controls in different gestational age groups. The p-value <0.05 was considered statistically significant.

RESULTS

The mean age of cases was 23.6±3.2 years and the mean age of controls was 23.8±3.2 years. Whereas the mean gestational age of cases was 12.1±3.3 weeks and that of controls was 12.3±2.9 weeks. In terms of socio-demographic features there was no significant difference between cases and controls [Table/Fig-2].

VEGF level- The mean serum VEGF in cases was 30.65±9.41 pg/mL whereas in controls it was 64.55±16.67 pg/mL. Repeated Measure ANOVA test was applied followed by Tukey's test and the critical difference was found to be 4.33. In the data the critical difference was more than 4.33 with p-value <0.001 in all the gestational age groups [Table/Fig-3]. So serum VEGF level was significantly low in TA patients compared to controls regardless of gestational age.

Sociodemographic characteristics	Control	Cases	p-value
Age (in years)	23.8±3.2	23.6±3.2	0.78
Religion			
Muslims	12 (15%)	10 (12.5%)	0.803
Hindu	28 (35%)	30 (37.5%)	
Education			
Primary school	5 (6.25%)	5 (6.25%)	0.822
High school/Intermediate	32 (40%)	31 (38.75%)	
Graduate	3 (3.75%)	4 (5%)	
Dietary habits			
Vegetarian	24 (30%)	22 (27.5%)	0.820
Non-vegetarian	16 (20%)	18 (22.5%)	

[Table/Fig-2]: Sociodemographic features of cases and controls. Chi-square test was used

Gestational age in weeks	VEGF levels in cases (pg/mL)	VEGF levels in controls (pg/mL)	p-value	Tukey's test
6-10	30.85±6.48	46.08±17.51	<0.001	Significant
11-15	31.60±11.95	75.52±2.89	<0.001	Significant
16-20	27.57±5.09	66.17±1.17	<0.001	Significant
6-20	30.65±9.41	64.55±16.67	<0.001	Significant

[Table/Fig-3]: Comparison of mean levels of VEGF in cases and controls.

PlGF level- Mean serum PlGF in TA patients was 263.54±68.108 pg/mL compared to 341.83±112.704 pg/mL in controls. Critical difference was 40.71 in repeated ANOVA followed by Tukey's test. Considering this PlGF level was found to be significantly lower in TA patients of all the gestational age group except in patients of 16-20 weeks of gastational age [Table/Fig-4]. The data obtained did not show any trend in the VEGF and PlGF levels with increasing gestational age in both cases and controls.

Gestational age in weeks	PlGF in cases in pg/mL	PlGF in controls in pg/mL	p-value	Tookies test
6-10	238.10±76.855	363.57±103.581	<0.001	Significant
11-15	279.05±67.559	347.55±125.122	<0.001	Significant
16-20	266.45±40.876	274.70±62.702	0.34	Non-significant
6-20	263.54±68.108	341.83±112.704	<0.001	Significant

[Table/Fig-4]: Comparison of mean PlGF levels in cases and controls.

DISCUSSION

In this study, the mean serum levels of VEGF in cases was 30.65±9.41 pg/mL whereas in controls was 64.55±16.67 pg/mL and the p-value was <0.001 in all the gestational age groups; hence the lowering in levels are significant for all age groups. Whereas mean serum levels of PlGF in threatened abortion cases was 263.54±68.108 pg/mL and in controls was 341.83±112.704 pg/mL. The lowering in PlGF value was statistically significant for the gestational age groups of 6-10 and 11-15 weeks but for the gestational age group of 16-20 weeks the change in value was not statistically significant.

The results of this study are consistent with that of Patrelli TS et al.,; they found a mean VEGF-A value of 25.64±56.17 pg/mL and a mean PlGF value of 167.14±264.72 pg/mL in the subgroup with TA and bleeding and a mean VEGF-A value of 36.98±143.69 pg/mL and a mean PlGF value of 201.88+ -282.00 pg/mL in the subgroup with uncomplicated pregnancy [10].

However, in a case control study by Keskin U et al., at 8th week of gestation the serum levels of VEGF-A were significantly elevated in pregnant women with TA (39.10 ng/mL) compared to pregnant women without first trimester bleeding (5.24 ng/mL) at 8 weeks of gestation but there was no significant increase in PlGF levels in pregnant women with TA. PlGF in TA was 20.80 ng/mL whereas in controls the level was 20.16 ng/mL [1].

Muttukrishna S et al., found that PlGF levels were lesser in TA patients with a consequent miscarriage (44% decrease) as compared to TA patients who had term live birth. Also, TA patients with subsequent miscarriage had lower levels of PlGF (42% decrease) as compared to asymptomatic pregnant women [5].

Elkholi DGEY and Nagy HM also found that concentration of PlGF was markedly reduced in maternal serum of TA (due to impaired placentation) resulting in foetal loss as compared to normal pregnant women. They also found that the incidence of adverse pregnancy outcomes were significantly higher in TA ($p<0.05$) than controls [3]. Another large prospective study on more than 1000 pregnant female reported an inverse relationship between increasing maternal serum PlGF and rate of spontaneous abortion [4]. Ziganshina MM et al., described imbalance of several angiogenic factors including VEGF and PlGF in early pregnancy loss [12]. Increased maternal serum PlGF levels has also been shown to be associated with reduced risk of late miscarriage [13].

The levels of VEGF and PlGF were studied with different gestational ages but any trend could not be elicited. Patrelli TS et al., found a statistically significant correlation between VEGF and PlGF with gestational age. They found an increasing trend for PlGF and a decreasing trend for VEGF [10].

Limitation(s)

The present study evaluated the values of VEGF and PlGF in patients of TA and normal pregnant women. It provides a platform for further research to suggest if these markers have any clinical and prognostic significance in patients with TA. Limitations of this study was small sample size and that the patients were not followed-up for the outcome of pregnancy.

CONCLUSION(S)

In the present study maternal serum VEGF and PlGF level was found to be significantly lower in patients with TA compared to uncomplicated pregnant women regardless of gestational age. But it will be too premature to draw a robust interpretation on the role of these markers in TA patients. Further, large scale studies are required to see their role as biomarkers or in prognostic stratification in these patients.

Declaration: This study was presented orally in the Association of Obstetricians and Gynaecologists of Delhi 2017 conference.

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