



## ASSESSMENT OF MATERNAL SERUM LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND PLACENTAL GROWTH FACTOR IN THREATENED ABORTION WITH OR WITHOUT SUB-CHORIONIC HEMATOMA

### Gynaecology

**Alpana Singh**

Associate Professor, Department of Obstetrics & Gynaecology, University College of Medical Sciences, and GTB Hospital, Delhi

**Sugandha Dev\***

Senior Resident, Department of Obstetrics & Gynaecology, University College of Medical Sciences, and GTB Hospital, Delhi \*Corresponding Author

**BD Banerjee**

Professor, Department of Biochemistry, University College of Medical Sciences and GTB Hospital, Delhi

### ABSTRACT

**Objective :** Assessment of maternal serum levels of vascular endothelial growth factor and placental growth factor in threatened abortion cases with and without sub-chorionic hematoma.

**Materials and Methods:** 3 ml of peripheral blood sample was collected from each case. The plasma was separated and quantification of VEGF and PIGF was done using ELISA kits.

**Results:** The VEGF levels were  $24.90 \pm 16.16$  pg/ml if sub-chorionic hematoma was present and the levels were  $31.29 \pm 9.59$  pg/ml in the absence of hematoma. Hence the VEGF level was significantly decreased in the presence of sub-chorionic hematoma. Whereas the PIGF value was  $281.76 \pm 57.95$  pg/ml in the presence of sub-chorionic hematoma and the value was  $257.46 \pm 71.01$  pg/ml in the absence of hematoma with the p-value 0.29.

### KEYWORDS

Threatened abortion, VEGF, PIGF, SCH

### INTRODUCTION

Threatened abortion is the most common complication of pregnancy which occurs in about 20% of the cases and 15-20% end up with spontaneous abortion.<sup>1</sup> Threatened abortion (TA) is described as vaginal bleeding with or without pelvic pain upto 20 weeks when the cervical os is closed and the ultrasonographic evaluation shows a viable fetus.<sup>2</sup>

TA is generally not associated with serious morbidity and mortality and if spontaneous abortion does not follow then the risk for later adverse pregnancy outcomes such as preterm birth, premature rupture of membranes, low birth weight, fetal growth restriction, placental abruption and caesarean delivery increases.<sup>2</sup> Unlike other types of abortion, the pathophysiological mechanisms resulting in TA are not fully understood.<sup>1</sup>

In normal pregnancies, the earliest stages of placental development take place in a low oxygen (O<sub>2</sub>) environment. This physiological hypoxia of the early gestational sac protects the fetus against the teratogenic effects of O<sub>2</sub> free radicals and stimulates cytotrophoblast proliferation while inhibiting trophoblast invasion. As the second trimester approaches there occurs a rise in pO<sub>2</sub> as maternal blood flow starts; triggering trophoblast from the proliferative state to invasive state leading to secondary wave of trophoblast invasion of maternal spiral arterioles. This results in a high flow, low impedance uteroplacental circulation. This coordination of trophoblast and endothelial cell development, proliferation, invasion and differentiation is mediated by locally acting growth factors which are probably regulated by oxygen tension and mechanical stimuli.<sup>3,4,5</sup>

Vaginal bleeding during early pregnancy most commonly originates from placenta and is an outcome of defective placentation which results in synthesis and release of altered angiogenic and antiangiogenic factors by placenta in the maternal circulation.<sup>6</sup> Regulation of normal placentation involves the interaction of various pro- and anti-angiogenic factors, angiopoietins and matrix metalloproteinases. VEGF-A (vascular endothelial growth factor-A), PIGF (placental growth factor) and their receptors are expressed in human placenta throughout gestation and placenta differentiation and maturation of villous vasculature requires VEGF-A and PIGF.<sup>8,9</sup> VEGF family is known to regulate placental angiogenesis and maternal spiral artery remodelling whereas PIGF is an important paracrine regulator of decidual angiogenesis and autocrine mediator of trophoblast function.<sup>10,11</sup>

In early pregnancy failure, the development of the placento-decidual interface is severely impaired leading to premature and excessive entry

of maternal blood inside the placenta which has two effects on the villous tissue. First a direct mechanical effect of large inter-villous blood thrombi which progressively embeds the villi and secondly an indirect O<sub>2</sub>-mediated oxidative damage leading to apoptosis and necrosis of the villous trophoblast. This leads to placental degeneration with loss of syncytiotrophoblast function and detachment of the placenta from the uterine wall. The loss of syncytiotrophoblast function may result in lowering of PIGF and VEGF.<sup>7,12,13</sup>

The premature perfusion of the intervillous space results in sub-chorionic haemorrhage (SCH).<sup>14</sup> Shallow trophoblast invasion and impaired angiogenesis resulting in friable blood vessels may be a predisposing factor of sub-chorionic haemorrhage, as well as adverse outcomes. The presence of a hematoma may create an area of weakness, where further separation of the placenta from the uterine wall may occur, resulting in placental abruption which is more common with retroplacental type of haemorrhage. The presence of an SCH and detachment of the gestational sac from the endometrium may result in miscarriage.

There are no studies in literature that have investigated the levels of VEGF, PIGF in the serum of patients with TA with and without sub-chorionic hematoma. This study aims to assess the maternal serum levels of VEGF and PIGF in cases of threatened abortion with and without SCH to understand the pathophysiology of threatened abortion.

### METHODS

This was a case control study conducted in the Department of Obstetrics and Gynecology in collaboration with the Department of Biochemistry at University College of Medical Sciences and Guru Teg Bahadur Hospital Delhi from November 2015 to April 2017.

The aim of the present study was to assess the maternal serum levels of VEGF and PIGF in cases of threatened abortion with and without SCH. Considering a range of VEGF in study of Ugr Keskin et al in cases of threatened abortion ( $6.57-163.56$  ng/ml) versus ( $0.84-15.08$  ng/ml) in uncomplicated pregnancy. The median of VEGF levels in cases of threatened abortion was  $39.10$  ng/ml and  $5.24$  ng/ml in uncomplicated pregnancy; which is making 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 were required.

In this study the preliminary results showed the levels of  $20 \pm 10$  ng/ml in cases and  $30 \pm 20$  ng/ml in controls. Considering these PIGF levels to estimate a difference of 10 units at alpha=5% and power=80% a sample of 40 cases were required. So for the present study 40

threatened abortion cases were enrolled.

**Selection of cases**

**Inclusion Criteria for cases**

- 1) 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
- 2) Ultrasound confirmed intrauterine pregnancy with or without choriodecidual haemorrhage, internal os closed and normal cervical length upto 2.5 cm

**Exclusion Criteria for cases**

- 1) Pregnant women of more than 20 weeks of gestation
- 2) H/o previous spontaneous abortion
- 3) H/o any previous pregnancy complication such as pregnancy induced hypertension, gestational diabetes mellitus, intrauterine growth restriction, intrauterine death, preterm labour, premature rupture of membranes
- 4) H/o any medical disorder such as diabetes mellitus, hypertension, asthma, antiphospholipid antibody syndrome, thyroid dysfunction, tuberculosis and other chronic disorder
- 5) Diagnosed uterine anomaly and cervical insufficiency or any uterine surgeries like myomectomy, septoplasty, adhesiolysis for Asherman's syndrome except previous caesarean section.

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

3 ml of peripheral blood sample was collected from each case in EDTA vial. The plasma was separated by centrifuging at 2000 rpm for 10 minutes and stored at -80 degrees Celsius till further analysis. Quantification of VEGF and PIGF was done by using commercially available ELISA kits. The data was analysed using unpaired t test.

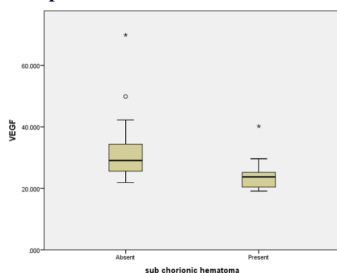
**OBSERVATION AND RESULTS**

The VEGF levels were  $24.9 \pm 6.16$  pg/ml if sub-chorionic hematoma was present and the levels were  $31.29 \pm 9.59$  pg/ml in the absence of hematoma; hence the VEGF levels were decreased in the presence of sub-chorionic hematoma. Whereas PIGF levels were increased if there was presence of SCH. The PIGF value was  $281.76 \pm 57.95$  pg/ml in the presence of sub-chorionic hematoma and the value was  $257.46 \pm 7.01$  pg/ml in the absence of hematoma. Unpaired T test was applied and the p-value was 0.02 for the VEGF Levels ; hence the decrease in VEGF levels in TA cases with SCH with respect to TA cases without SCH was statistically significant, however the p-Value for PIGF was 0.29 and hence the decrease in PIGF was not statistically significant (Table 1, Fig. 1,2).

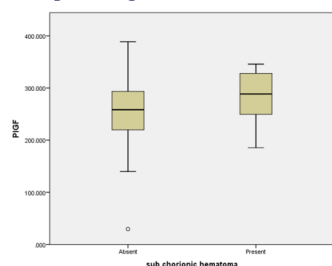
**Table 1: Mean Serum Levels Of VEGF and PLGF In The Presence Of Sub-chorionic Hematoma**

Sub-chorionic hematoma	Present	Absent	p-value
VEGF levels ( pg/ml)	$24.9 \pm 6.16$	$31.29 \pm 9.59$	0.02
PIGF levels ( pg/ml)	$281.76 \pm 57.95$	$257.46 \pm 71.01$	0.29

**Fig. 1: Box Plot Graph Of VEGF Level In Sub-chorionic Hematoma**



**Fig. 2: Box Plot Graph Of Plgf Level In Sub-chorionic Hematoma**



**DISCUSSION**

Threatened abortion is one of the most common complication of pregnancy.<sup>1</sup> TA is generally not associated with serious morbidity and mortality and if miscarriage does not occur after early bleeding, then also there is a risk for later adverse pregnancy outcomes.<sup>2</sup> During early pregnancy vaginal bleeding most commonly originates from placenta and is an outcome of defective placentation. Hence, the result is synthesis and release of altered angiogenic and antiangiogenic factors by placenta in the maternal circulation such as VEGF and PIGF.

In early pregnancy failure, the development of the placento-decidual interface is severely impaired leading to premature and excessive entry of maternal blood inside the placenta leading to SCH which has two effects on the villous tissue. First a direct mechanical effect of large inter-villous blood thrombi which progressively embeds the villi and secondly an indirect O2-mediated oxidative damage leading to apoptosis and necrosis of the villous trophoblast. This leads to placental degeneration with loss of syncytiotrophoblast function and detachment of the placenta from the uterine wall. The loss of syncytiotrophoblast function may result in lowering of PIGF and VEGF.<sup>7,12,13</sup>

In our study the VEGF Levels were decreased if there was presence of subchorionic hematoma and it was statistically significant. Whereas PIGF levels were increased if there was presence of SCH; however the p-Value is 0.22 and hence was not statistically significant.

There are no studies in the literature which have measured the maternal serum levels of VEGF and PIGF in the presence of subchorionic hematoma in threatened abortion patients.

However in a retrospective cohort study by sukur et al. Patients with threatened abortion (n=242) were followed. The inclusion criteria were hospitalization due to threatened abortion, singleton pregnancy, gestational age <20 weeks, and they were followed up until the end of the pregnancy. The study group consisted of 44 patients with SCHs observed on ultrasonography, and the control group consisted of 198 patients without SCHs. The incidence of SCH among patients with threatened abortion was 18.2% (44/242) and found that ultrasonographically detected subchorionic hematoma increases the risk of miscarriage in patients with vaginal bleeding and threatened abortion during the first 20 weeks of gestation. However, it does not affect the pregnancy outcome measures of ongoing pregnancies.<sup>14</sup>

Gianpaolo Maso et al found that the overall risk of adverse pregnancy outcome was increased by 2.4 times when the subchorionic hematoma was diagnosed before 9 weeks of gestation.<sup>15</sup> Similarly in a study by Jaishree banniya et al done in 144 patients of threatened abortion found that patients who presented with bleeding and had subchorionic hematoma <4cm<sup>2</sup> had less incidence of spontaneous abortion (16.66%) as compared to 25% in group with hematoma >4cm<sup>2</sup>.<sup>16</sup>

**CONCLUSION**

Placental degeneration in presence of SCH results in detachment of the placenta from the uterine wall and loss of syncytiotrophoblast function hence lowering of PIGF and VEGF. In our study we observed a significant lowering of VEGF whereas there was an increase in PIGF which was not significant. Previous studies indicate that the presence of SCH in TA leads to adverse pregnancy outcomes .The addition of VEGF levels with presence of SCH in threatened abortion may add value in predicting the adverse outcome and hence help in counseling the threatened abortion patient in a holistic way. This needs further studies in future establishing the levels of VEGF and PIGF with pregnancy outcomes.

**REFERENCES**

1. Keskin U, Ulubay M, Dede M, Ozgurtas T. The relationship between the VEGF/sVEGFR-1 ratio and threatened abortion. Arch Gynecol Obstet 2015;291(3):557-61.
2. Cunningham FG, Leveno KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL, et al, eds. William's Obstetrics, 24th ed. Philadelphia: McGraw Hill; 2015:354-55.
3. Sotiriadis A, Papatheodorou S, Makrydimas G. Threatened miscarriage: evaluation and management. BMJ 2004;329(7458):152-55.
4. Makrydimas G, Sebire NJ, Lolis D, Vlassis N, Nicolaidis KH. Fetal loss following ultrasound diagnosis of a live fetus at 6-10 weeks of gestation. Ultrasound Obstet Gynecol 2003;22:368-72.
5. Johns J, Hyett J, Jauniaux E. Obstetric outcome after threatened miscarriage with and without a hematoma on ultrasound. Obstet Gynecol 2003;102:483-87.
6. Elkholi DGEY, Nagy HM. Maternal serum concentrations of angiogenic and antiangiogenic factors in threatened miscarriage at 7-12 weeks' gestation and the risk of adverse pregnancy outcomes. Middle East Fertil Soc J 2013;18:208-213.

7. Muttukrishna S, Swer M, Suri S, Jamil A, Calleja-Agius J, Gangooly S, et al. Soluble Flt-1 and PlGF: new markers of early pregnancy loss? *PLoS One* 2011;6(3):e18041.
8. Kutluer G, Ertargin P, Sankaya E. Low VEGF expression in conceptus material and maternal serum AFP and b-HCG levels as indicators of defective angiogenesis in first-trimester miscarriages. *J Turkish-German Gynecol Assoc* 2012;13:111-17.
9. Tseng JJ, Chou MM, Wen MC, Hsu SL. Differential expression of VEGF, PlGF and their receptors in placentae from pregnancies complicated by placenta accreta. *Placenta* 2006;27:70-78.
10. Andraweera PH, Dekker GA, Roberts CT. The vascular endothelial growth factor family in adverse pregnancy outcomes. *Hum Reprod Update* 2012;18(4):436-57.
11. Plaisier M, Dennert I, Rost E, Koolwijk P, van Hinsbergh VW, Helmerhorst FM. Decidual vascularization and the expression of angiogenic growth factors and proteases in first trimester spontaneous abortions. *Hum Reprod* 2009;24(1):185-97.
12. Jelkmann W. Pitfalls in the measurement of circulating vascular endothelial growth factor. *Clin. Chem* 2001;47:617-23.
13. Kou R, SenBanerjee S, Jain MK, Michel T. Differential regulation of vascular endothelial growth factor receptors (VEGFR) revealed by RNA interference: Interactions of VEGFR-1 and VEGFR-2 in endothelial cell signaling. *Biochemistry* 2005;44:15064-73.
14. Sukur YE, Goc G, Kose O, Acmaz G, Ozmen, Atabekoglu CS. The effects of subchorionic hematoma on pregnancy outcome in patients with threatened abortion. *J Turk Gynecol Assoc* 2014;15:239-42.
15. Maso G, De Seta F, Sartre A. First-trimester intrauterine hematoma and outcome of pregnancy. *Obstet Gynaecol* 2005;105:339-44.
16. Bamanija J, Shah H, Singh P, Doshi H, Shah M, Ladola HM. Pregnancy outcome in patients with threatened abortion and abnormal early sonography markers: a prospective study. *International Journal of Biomedical and Advance Research* 2015;6(8):580-83.