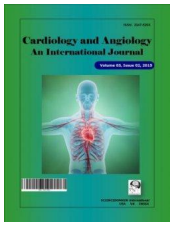

Cardiology and Angiology: An International Journal

7(4): 1-9, 2018; Article no.CA.43655
ISSN: 2347-520X, NLM ID: 101658392

Vascular Endothelial Growth Factor Study of Placenta in Preeclampsia

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Authors' contributions

This work was carried out in collaboration between all authors. Author JR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BMS and NS managed the analyses of the study. Author NS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CA/2018/43655
Editor(s):
(1) Dr. Sam Said, Department of Cardiology, Hospital Group Twente, Hengelo, The Netherlands.
(2) Dr. Thiago Andrade de Macedo, Hypertension Unit, Cardiology Division Heart Institute (InCor), Brazil.
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Reviewers:
(1) Sreelatha S. Ramesh, International Medical University, Malaysia.
(2) Panagiotis Tsikouras, Democritus University of Thrace, Greece.
(3) Anonymous, Bulgarian Academy of Sciences, Bulgaria.
Complete Peer review History: http://www.sciencedomain.org/review-history/26451

Original Research Article

Received 01 July 2018
Accepted 17 September 2018
Published 29 September 2018

ABSTRACT

Background: Preeclampsia is defined as new onset hypertension and albuminuria in previously normotensive pregnant women after 20 weeks of pregnancy. There is no cure; management is reliant on a structured antenatal surveillance programme and antihypertensives. Recent advances in immune histochemistry study of placenta have elucidated an increased Vascular Endothelial Growth Factor (VEGF) expression in various placental bed disorders like recurrent pregnancy loss, preeclampsia, fetal growth restriction, preterm and abruption placenta. Increased release of VEGF family proteins has been attributed to atherosis and placental hypoxia. However, some studies have found normal VEGF concentrations in placenta in these disorders of feto-maternal interphase.

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Objectives: The study aims to analyse the VEGF expression in placental biopsy from preeclampsia and normotensive pregnancies.
Materials and Methods: This prospective study involved the gross and immune histology examination of human placenta after 31-40 weeks gestation period in 20 singleton preeclampsia pregnancies. Twenty placentas of normotensive pregnancies were taken as controls.
Results: In the present study, VEGF density was more in the placentas from preeclampsia pregnancies as compared to placenta from a normal pregnancy. The mean weight of placenta was smaller in preeclampsia group. Additionally, the fetal capillaries were also small in diameter and lumen was collapsed. The pulsatility index of uterine artery supplying the placenta was also higher in preeclampsia pregnancies. The high velocity blood flow can mechanically damage the tender fetal villi floating in the intervillous space. This damage collapses the fetal capillaries as evidenced by the smaller diameter of fetal capillaries in the placental biopsy.
Conclusion: Placental hypoxia in cases of preeclampsia is a potent stimulus for VEGF expression. Nevertheless, the increased VEGF expression should be seen in the light of collapsed fetal vessels in a small placenta.

Keywords: Immune; histochemistry; vascular endothelial growth factor; placenta; preeclampsia.

ABBREVIATIONS

VEGF : Vascular Endothelial Growth Factor
JZ : Junctional Zone
VPF : Vascular Permeability Factor
sFLT : Soluble Fms Like Tyrosine kinase
sENG : Soluble Endoglin
PLGF : Placental Growth Factor
mRNA : Messenger RNA
CRL : Crown Rump Length
HELLP : Hemolysis Elevated Liver Enzymes Low platelet
SABC : Streptavidin-Biotin-Complex
DAB : 3, 3´-Diamniobenzidine
BMI : Body mass Index
IUGR : Intrauterine Growth Restriction
CTG : Cardiotocogram

1. BACKGROUND

Preeclampsia affects 7-10% of all pregnancies and is a leading cause of maternal morbidity and mortality [1]. It complicates 1 in 2,000 pregnancies in the UK and 1 in 100 in developing countries [1]. Preeclampsia has multiple system involvement defined by persistent hypertension associated with albuminuria, thrombocytopenia, impaired liver function, progressive renal insufficiency, pulmonary oedema and cerebral oedema [2,3]. The basic pathology is placental ischemia leading to release of sFLT and sENG which bind the VEGF, PLGF and endoglin. Deprived of these angiogenic factors, there is, hypoxia, reperfusion injury and generation of reactive oxygen species which gradually lead to the release of cytokines, lipid peroxidises and syncytiotrophoblast micro-fragments from the placenta into the maternal circulation [4].

In preeclampsia, two stages of vascular dysfunction exists [5]. In the first stage, there is a decreased ratio of proangiogenic and antiangiogenic factors leading to placental ischemia, and subsequently, after 20 weeks, there is hemodynamic maladaptation to the increased cardiac output and blood volume during pregnancy. This leads to the defective placentation. Defective placentation processes include decreased migration of trophoblastic cells into the endometrium and junctional zone myometrium, defects in spiral artery remodelling and proliferation of fetal floating and anchoring tertiary stem villi in the placental sinuses. There is an acute atherosis and, spiral arteries are occluded by fibrinoid material and surrounded by foam cells. Acute atherosis and placental thrombi are also seen in low birth weight babies. After 20 weeks, a clinically evident maternal syndrome of hypertension, oedema and proteinuria develops.

As early as 9 weeks, the uterine decidual natural killer cells secrete vascular endothelial growth factor (VEGF), placental growth factor (PLGF) and angiopoietins [6]. During normal pregnancy, the placenta expresses the VEGF family angiogenic factors, but the studies about the source of VEGF provide contradictory evidence. Some studies have shown that VEGF mRNA is expressed in villous and extravillous trophoblast, Hofbauer cells and maternal decidual cells, throughout the gestation period [7,8,9,10,11]. Several studies suggested that VEGF m RNA is expressed villous mesenchyme, decidual macrophages, decidual glands but not in the trophoblast [9]. In the first twelve weeks of gestation, PLGF is mainly expressed in extravillous cells within the maternal decidua, but

near term the expression is more in villous trophoblast [9,10]. The inconsistent findings might be attributed to the probes used in-situ hybridisation techniques, which may cross, react with PLGF and provide false positive results [9].

The altered ratio of proangiogenic and antiangiogenic factors leads to impaired vacuolation and disorganisation of vascular intima and endothelial cells. In Junctional Zone myometrium, since the natural killer cells are absent, the presence of interstitially invading trophoblast may help the release of VEGF and angiopoietins [6]. VEGF also called as VPF or vascular permeability Factor. As there is no angiogenesis in embryo, the endometrial VEGF is postulated to be involved in fluid transport in the embryo and increased endometrial embryo cell adhesion, thereby favouring implantation.

After 12 weeks, the placenta expresses the VEGF family angiogenic growth factors [12]. VEGF is an active protein growth factor, promotes endothelial cell growth, angiogenesis and cell migration. However, it also increases the capillary permeability. Excessive expression of VEGF in trophoblast, stromal cells, Hofbauer cells, and fetal tertiary anchoring and floating stem villi has been documented in various animal and human studies [13,14]. However, the results were inconsistent in various studies as the probes used for VEGF cross-react with PLGF in the trophoblastic layer, resulting in false positive results [15,16]. VEGF expression in Hofbauer cells, is responsible for the activation of the endothelial precursors to form angiogenic cords of fetal villi is also significant [17,18].

Hence this study was designed to study the VEGF expression in placentas of preeclampsia and normotensive pregnancies.

2. MATERIALS AND METHODS

This prospective study involved immune histochemistry of placenta with high uterine artery pulsatility index and Preeclampsia delivered at 33-40 weeks gestation period in women with singleton pregnancies. This study was approved by the ethical and research board. All women with no significant fetal anomaly were offered for uterine artery Doppler evaluation. Written consent was obtained in all cases. A first trimester scan was done to measure CRL (Crown Rump Length) to date the pregnancy in all cases.

The research was included in 20 preeclampsia pregnancies as the study group. Control group had 20 pregnant women. Permission was obtained from the Ethical Committee and Department of Pathology and Department of Obstetrics and Gynecology at Saveetha Medical College and Hospital, Chennai, India. Pregnant women were recruited between 1 April 2015 and 31 December 2016 after getting written informed consent from participants in local language. Multiple Pregnancies and pregnancies with congenital anomalies were excluded. Detailed maternal factors like age, gestational age, parity, pre-pregnancy body mass index, previous low birth weight, haemoglobin levels, chronic hypertension, gestational diabetes and previous preeclampsia were recorded subsequently.

2.1 Fetal Ultrasound and Uteroplacental Doppler Study

The ultrasound machines used for this study were PHILIPS HD11XE (Acuson, Mountain View, CA, USA); GE LOGICS7 Expert; Siemens Sonoline Acuson X150 (Siemens). Inclusion criteria were Preeclampsia with high pulsatility index of uterine artery (>1.55). Exclusion criteria were women with chronic hypertension, renal disease, and diabetes mellitus and HELLP syndrome (Hemolysis Elevated Liver Enzymes Low platelet).

Pulsed wave Doppler examination of uterine artery in the longitudinal scan was conducted to obtain three similar consecutive waveforms. The same was repeated for the contralateral uterine artery, and the mean pulsatility Index (Maximum-Minimum velocity/Mean velocity) of the two vessels was estimated. Pulsatility index rather than resistivity index was considered because PI describes the shape of the velocity waveform much better as it includes the area below the curve into the formula. Presence or absence of an early diastolic notch was recorded.125 Hz high pass filter was used to eliminate signals from slowly moving tissues. The curved transducer (3.5-or 5-MHz) had spatial peak temporal average intensities <100 mW/cm^2. Recordings for measurements were recorded in the absence of fetal breathing movements and fetal heart rate between 120-160 beats per minute. The angle between the ultrasound beam and the direction of blood flow was always less than 30°.

2.2 Placental Histology and Immuno-staining

Placental problems like infarcts, retroplacental calcifications, small placenta, and premature separation were also reported. Placenta from 20 normotensive, nonproteinuric pregnant women with standard pulsatility index (<1.55) of uterine artery was studied as a control group. On the day of delivery, the placenta was weighed and 12 full thickness blocks of placenta were made from centre and periphery. The blocks were incubated in 4% buffered formalin for 12 hours and sections were taken at 5 microns spacing. Heat mediated antigen retrieval was carried out by incubating in citrate buffer (pH 6, epitope retrieval solution) for 20 minutes. The specimen was then incubated with 1microgram /ml Rabbit anti VEGF A antibody (MOOO45-1) overnight at 4°C. Biotinylated goat antirabbit IgG was used as secondary antibody, and sample was then incubated at 37°C for 30 minutes. The tissue section was developed by using Streptavidin-Biotin-Complex (SABC) with DAB (3, 3´-Diamniobenzidine) as the chromagen. A blinded single observer examined ten fields. Placental specimens were scored for VEGF staining in trophoblast, stromal cells, vessel walls and Hofbauer cells.

3. RESULTS

Table 1 summarises the maternal history variables associated with preeclampsia and normotensive pregnancies. The mean age of preeclampsia pregnancy is 24.29 (1.22) as compared to normal pregnancy i.e., 23.20 (0.94). The average BMI of patients in preeclampsia group is 23.5 while mean body mass index in normal pregnancy group was 23.3. Thus the groups were comparable in age and BMI.

The mean gestational age at delivery was 36.62 in preeclampsia and 38.5 in normal pregnancy. The average weight of placenta in preeclampsia group was 463.5 (30.4) grams while in normal pregnancies the average placental weight was 640 (54.6) grams. The average birth weight was 3040 grams in normal pregnancy while in preeclampsia the average birth weight was 2378 grams. The mean systolic and diastolic pressures in normal pregnancy were 112.6/65 while in preeclampsia the values were significantly high at 165.4/96.2. The Mean uterine artery pulsatility Index in preeclampsia group was 1.644 while in normal pregnancy group the uterine artery pulsatility index was 0.944.

Gross pathological study of placenta in Normotensive and Preeclampsia pregnancies are summarised in Table 2. The placental examination was normal in 2/20 for preeclampsia pregnancies and 15/20 for normal pregnancies. In preeclampsia, Hofbauer cells (placental lymphocytes) were also seen.

Table 3 brings up the VEGF immune histochemistry findings in placenta from preeclampsia and normotensive pregnancy in 100X and 400X magnification.

In the present study, Hofbauer cells were not stained for normal placentas, but they were stained for the preeclampsia placenta. This disparity could be explained by the altered expression of Hofbauer cells in normal, and preeclampsia pregnancies as these cells varies in number, phenotype in preeclampsia and normal pregnancies [17,18].

4. DISCUSSION

This study interrogates the VEGF expression in placenta from preeclampsia pregnancies. The immune histochemical study reveals that cells of VEGF were expressed in increased concentrations of preeclampsia in endothelial cells of anchoring and floating tertiary fetal stem villi in placental sinuses. However, the mean velocity and pulsatility index of blood flow in the uterine arteries supplying the uteroplacental sinuses was also high (Table 1).

Stromal cells and trophoblast cells did not show VEGF expression. Hofbauer cells with immune histochemical VEGF positive stain were seen in placenta from preeclampsia pregnancy. This finding is consistent by several other studies done on human placenta [19,20,21, 22,23].

The gestational age of delivery was lower in case of preeclampsia group as compared to normal pregnancy. This may be attributed to intense Doppler fetal surveillance and CTG done in preeclampsia pregnancies, which leads to early detection of hypoxic fetal circulation. Labour inductions and Caesarean sections were planned much prior to expected date of delivery [24]. Other placental bed disorders like recurrent pregnancy loss Preterm labour, IUGR and Abruption placenta have also been associated with increased VEGF expression [25,26,27].

Table 1. Clinical details of the study group values shown are arithmetic mean (SEM)

Clinical characteristic	Control group (n=20)	Preeclampsia (n=20)
Maternal Age (years)	23.20(0.94)	24.29(1.22)
BMI	23.3	23.5
Gestational Age at delivery (weeks)	38.5(0.41)	36.62(0.44)
Birth weight (g)	3040(58.2)	2378(110)
Placental Weight (g)	640(54.6)	463.5(30.4)
Primigravida (n)	12	10
Systolic Pressure (mm Hg)	112.6(1.2)	165.4(2.8)
Diastolic Pressure (mm Hg)	65(2.24)	96.2(1.5)
Mean Uterine artery Pulsatility Index	0.952(0.021)	1.644(0.024)

Table 2. Gross placental examination-preeclampsia group and control group

Placental examination	Preeclampsia	No preeclampsia
Calcifications	12/20(60%)	3/20(15%)
Infarctions	11/20(55%)	1/20(5%)
Placenta Praevia	2/20(10%)	1/20(5%)
Velamentous Insertion	1/20(5%)	0/20(0%)
Abruption Placenta	3/20(15%)	0/20(0%)

Table 3. Increased density of VEGF Reactivity is seen in preeclampsia as compared to control group control group. The vessels are small in diameter and collapsed

Immunohistochemical study	Trophoblasts	Vessels	Stromal cells	Hofbauer cells
Control Group	-	+	-	-
Preeclampsia Group	-	++	-	+

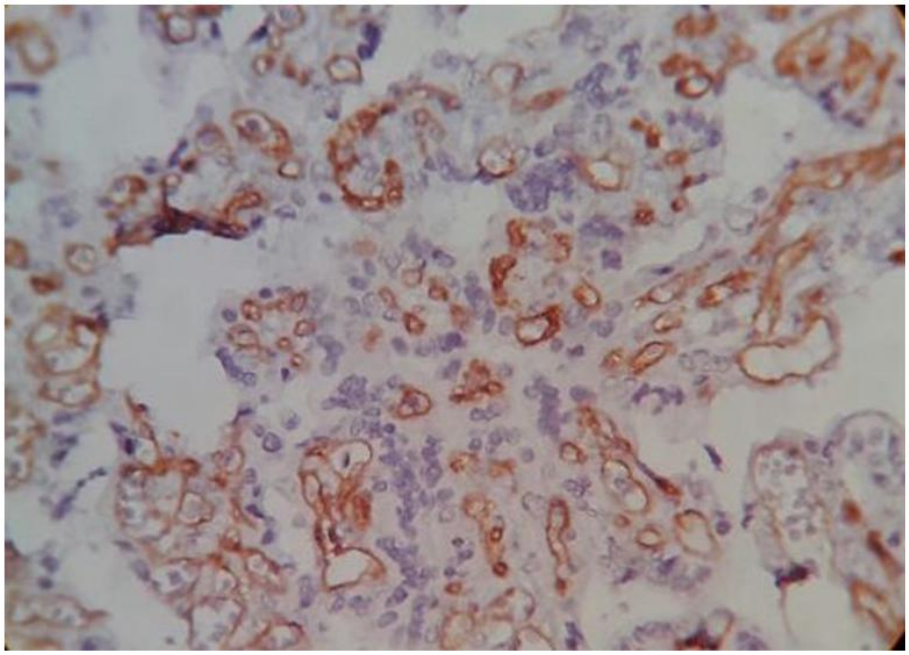

Fig. 1(a). VEGF immunostaining: Normal placenta in normal uterine artery pulsatility index of 37 weeks. Reactivity is observed in the endothelial cells of the Vessels (Original magnification X 100)

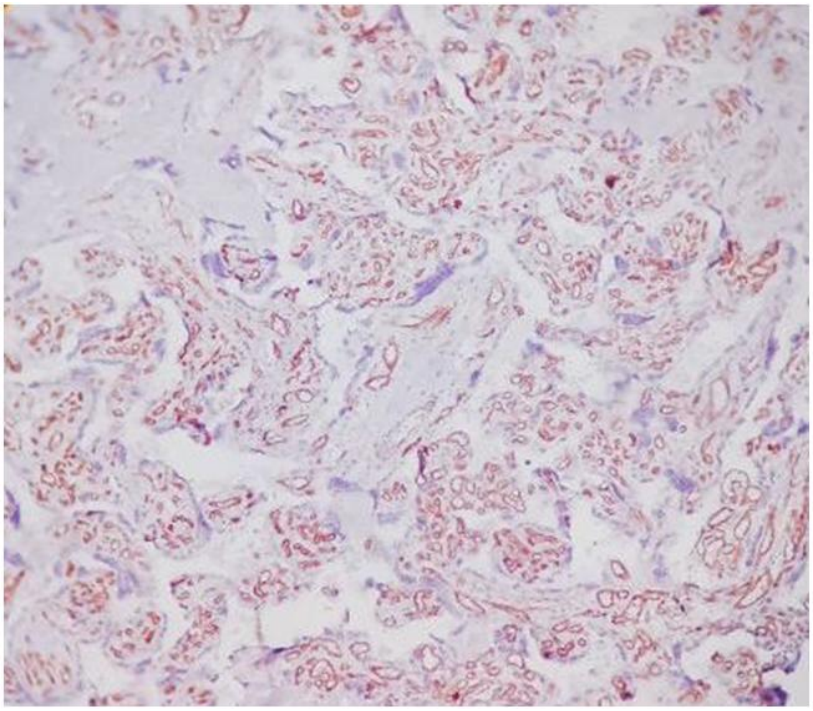

Fig 1(b). VEGF immunostaining: Preeclampsia, 37 weeks. Reactivity is seen in the endothelial cells of the Vessels (Original magnification X 100)

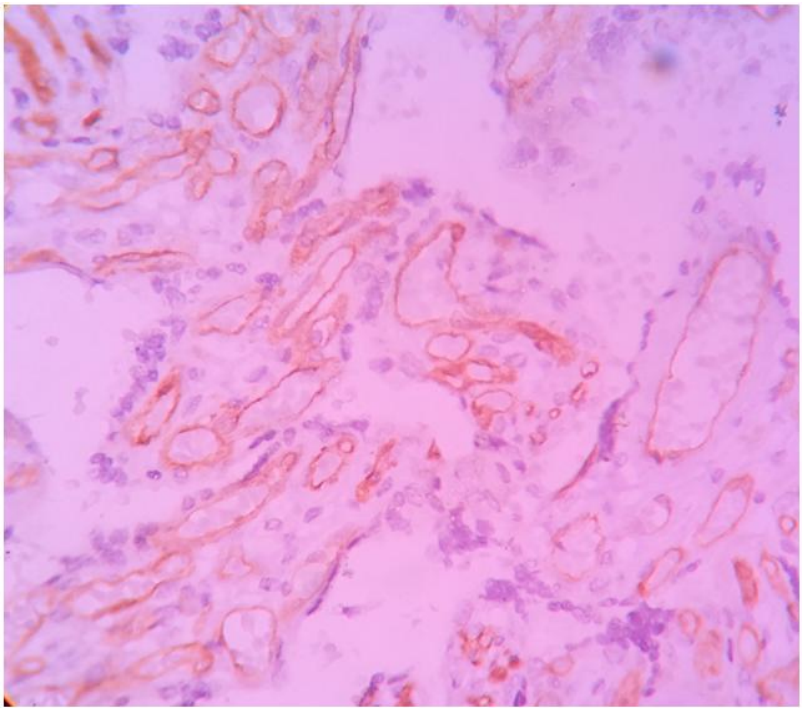

Fig. 2(a). VEGF immunostaining: Normal placenta in normal uterine artery pulsatility index, 37 weeks. Reactivity is observed in the endothelial cells of the vessels (Original magnification X 400)

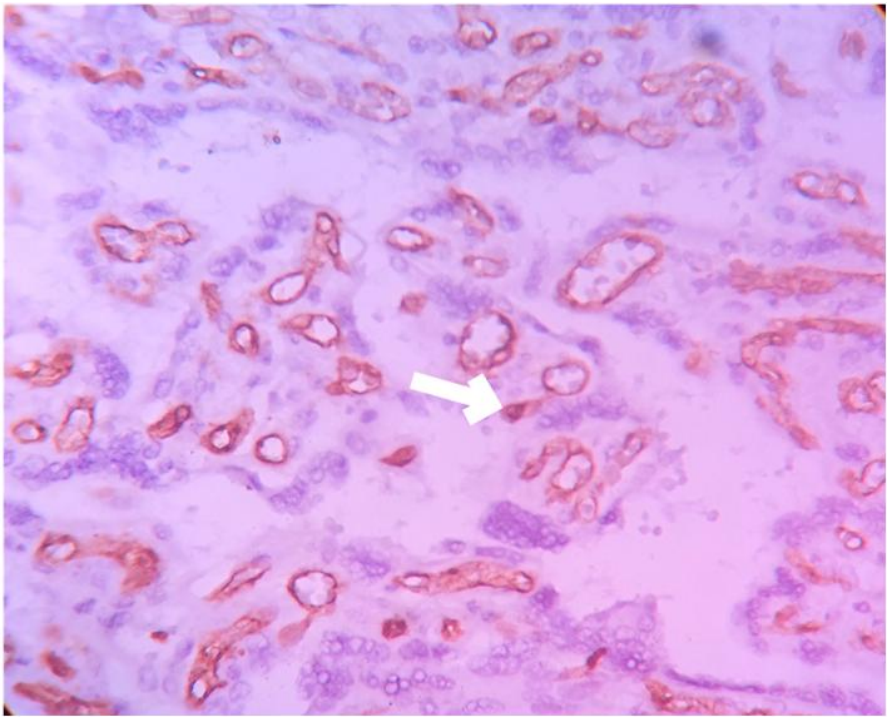

Fig. 2(b). VEGF immunostaining: Preeclampsia, 37 weeks. Reactivity is observed in the endothelial cells of the vessels and in Hofbauer cells (arrow) (Original magnification X 400)

The study also reveals that high pulsatility index of uterine artery is associated with smaller diameters of fetal tertiary stem villi. When the velocity of blood flow in uterine artery is high, the delicate fetal tertiary stem villi will get collapsed, as the blood is shot as a contrast to being sprinkled over the placental venous sinuses. Thus the collapse of fetal vessels can falsely lead to the expression of increased VEGF concentration density and expression as the placenta is small and fetal stem villi are shrunken. When the immune histochemical study is done on a single sectioned block in high power, this can appear as increased VEGF concentration.

Increased apparent expression of VEGF has also been supported by several other studies indicating that VEGF mRNA is primarily unaltered in preeclampsia and normal pregnancy [28,29,30,31,32]. This may provide indirect evidence of VEGF expression in foetal stem villi.

5. CONCLUSION

There is an apparent increased density of VEGF in small placenta of hypertensive pregnancies. Strict criteria should be used when performing VEGF immune staining. The immune histochemical VEGF concentration needs to be correlated in context of overall placental size. Biochemical and immune histochemical markers need to be deciphered in conjunction with biophysical markers like uterine artery pulsatility index.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DOI: 10.1080/14767058.2018.1462323

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26451