Immunohistochemical Evaluation of Vascular Endothelial Growth Factor-A (VEGF-A) in Human Placenta and its Role in Growth of Placenta and Fetus

Thesis submitted to

THE KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI

(KLE DEEMED UNIVERSITY)

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For the award of the degree of

Doctor of Philosophy

In the Faculty of

MEDICINE

By

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(Registration No: KLEU/Ph.D./16-17 /DO1216004)

Under the Guidance of

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AUGUST-2020

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ACKNOWLEDGMENT

I take this opportunity to thank each and everyone for their support and encouragement in completing my research work successfully.

I would like to express my sincere and heartfelt gratitude to my guide **Dr. Daksha Dixit,** Director, Academic Affairs, KLE Academy of Higher Education and Research, and Professor, Department of Anatomy, J. N. Medical College, Belagavi; for her guidance, constant support and critical evaluation throughout my work which helped me to complete my research work.

I am grateful to **Prof. (Dr.) C. K. Kokate,** Former Vice Chancellor and **Prof. (Dr.) Vivek A. Saoji,** Honourable Vice Chancellor, KLE Academy of Higher Education and Research, for giving me an opportunity to pursue my research work.

I also express my gratitude to **Dr. V. D. Patil,** Former Registrar and **Dr. V. A. Kothiwale**, Registrar, KLE Academy of Higher Education and Research for providing the requisite institutional facilities throughout my research.

I extend my gratitude to **Dr. R. S. Mudhol,** Dean, Faculty of Medicine and **Dr. (Mrs) Niranjana S. Mahantashetti,** Principal J. N. Medical College, for giving required institutional facilities to complete my research work.

I express my sincere thanks to **Dr. R. D. Virupaxi,** Former Head of the Department of Anatomy and **Dr. Shilpa M. Bhimalli**, Professor and Head of the Department of Anatomy, J. N. Medical College for their constant support and providing all the facilities to carry out my work. I also thank all the **staff members** of the department of Anatomy.

I sincerely thank **Dr. N. K. Tyagi,** Professor and Head of the Department of Epidemiology and Biostatistics, KLE Academy of Higher Education and Research, for guiding me throughout the work.

I thank the **Head of the Department** and all the **staff members** of Obstetrics and Gynaecology and all the **nursing staffs** of Obstetrics and Gynaecology Unit, Dr. Prabhakar Kore Charitable Hospital, Belagavi.

I express my heartfelt gratitude to **Dr. Adarsh Sanikop,** Assistant Professor, Department of Pathology, J. N. Medical College for the constant support and guidance.

I thank **Head of the Department** of Pathology, J.N. Medical College for providing institutional facilities to work.

My sincere thanks to **Dr. Sunil S Jalalpure,** Deputy Director and Scientist and all the **staff members** of Dr. Prabhakar Kore Basic Science Research Centre Belagavi for providing facilities to complete my work.

I thank **Professor and Head of the Department** and all the **staff members** of the Department of Oral Pathology and Microbiology, KLE V. K. Institute of Dental Sciences, Belagavi.

I thank my parents, brother and husband for their constant support and motivation.

Mrs. Vanitha

Date:

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LIST OF ABBREVIATIONS

AA	- Autoantibodies
Ang-1	- Angiopoietin-1
Ang-2	- Angiopoietin-2
APES	- 3,aminopropyltriethoxysilane
APH	-Antepartum hemorrhage
BV	- Blood vessels
CAM	- Chorioamnionitis
Cm	- Centimetre
Cm^2	- Centimetre square
C/S	- Cesarian Section
CT	-Cytotrophoblast
DAB	- 3', 3' Diaminobenzidine
DNA	- Deoxyribonucleic acid
Dpc	- days post coitus
DPX	- Dibutylphthalate polystyrene xylene
EC	- Eclampsia
EDTA	- Tris ethylenediaminetetraacetic acid
ELISA	- Enzyme-linked immunosorbent assay
EVT	- Extravillous trophoblast
Flt-1	- Fms like tyrosine kinase-1
GDM	- Gestational diabetes mellitus
GHP	- Gestational hypertension
Gm	- Gram
G/Dl	- Grams per decilitre

HC	- Hofbauer cells
HCG	- Human chorionic gonadotropin
H&E	- Hematoxyline and eosin
HELLP	- Hemolysis, Elevated Liver enzymes, Low Platelet count
HF	- hydropsfetalis
HIF-1	- Hypoxia inducible factor-1
HIF-1α	- hypoxia-inducible factor-1α
HRE	- Hypoxic response element
HRP	- Horseradish peroxidase
IUGR	- intrauterine growth restriction
KDa	- Kilodalton
KDR	- Kinase insert Domain Receptor
MAPK	- mitogen-activated protein kinase
MiR	- Micro ribonucleic acid
Ml	- Milliliter
Mm Hg	- Millimetre of mercury
mRNA	- Messenger ribonucleic acid
NK	- Natural killer cells
NO	- Nitric oxide
NRP-1	- Neuropilin -1
NRP-2	- Neuropilin -2
PE	- Pre-eclampsia
pg/mg	- Picogram per milligram
pg/ml	- Picogram per milliliter
pg/l	- Picogram per litre

pH	- Potential of Hydrogen
PIH	- Pregnancy-induced hypertension
PI3K	- Phosphoinositide 3-kinase
PlGF	- Placental Growth Factor
PROM	- Premature rupture of membranes
P Value	- Probability Value
RGB	- red, blue, green color
RT-PCR	- Reverse transcription polymerase chain reaction
SD	- Standard Deviation
sflt-1	- Soluble fms like tyrosine kinase-1
SGA	- Small for gestational age
STB	-Syncytiotrophoblast
sTNFR1	- Soluble tumor necrosis factor receptor-1
sVEGFR	- Soluble vascular endothelial growth factor rceptor
TB	- Trophoblast
TBS	- Tris buffer saline
TNFα-	- Tumor necrosis factor α-
VE-cadherin	- Vascular endothelial (VE)-cadherin
VEGF	-Vascular Endothelial Growth Factor
VEGFR	- VEGF receptor
VEGFR –	1– Vascular endothelial growth factor receptor -1
VEGFR –	2 – Vascular endothelial growth factor receptor -2
µm	- Micrometre

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ABSTRACT

Background: Vascular endothelial growth factor (VEGF) is an angiogenic growth factor having both angiogenic and vascular permeability elements. It helps in the development of first blood vessels and the formation of new vessels from the existing vessels. VEGF stimulates the proliferation of cytotrophoblast cells for the invasion of decidua and spiral artery remodelling. This process initiates uteroplacental circulation and helps in nutrition transport. It also stimulates nitric oxide, a vasodilator, reduces peripheral resistance of the maternal vessel and increases cardiac output thereby increases nutrition transport. These roles of VEGF in early pregnancy are associated with the growth of the placenta and fetus. But there is a lack of literature on its role in the growth in later stages of pregnancy and also discrepancies about its expression at term. Studying relationships between the VEGF level with placental and fetal parameters will explain its role in growth. This factor has been studied as a prediction marker for pre-eclampsia and intrauterine growth restriction pregnancies. However, the results varied. Therefore, a marker alone may not be beneficial in understanding the pathology. Therefore, there is a need to study its expression level and its relationship with growth parameters and factors influencing the VEGF expression, which will be beneficial in understanding the outcome of the complicated pregnancies.

Objective: To study the expression of VEGF in fetal growth by placental morphology and its immunohistochemistry.

Methodology: Placentae of 32 - 41+ weeks of gestation were collected from the Obstetrics and Gynaecology Unit, Dr Prabhakar Kore Charitable Hospital, Belagavi. The sample size was 385. Placentae of singleton pregnancy without any maternal

systemic disorders were included in the study. The placentae were observed for cord and membrane attachment. A central section of the placenta was taken and processed for immunohistochemical and Haematoxylin (H) and Eosin (E) staining. VEGF-A165 Mouse Monoclonal Antibody was used. The VEGF expression was observed in syncytiotrophoblast, blood vessels and Hofbauer cells. Depending on the expression, the intensity of staining was quantified from these cells in Image J Software.

Results: The expression of VEGF did not change from 32 - 40 weeks of gestation, but the expression was increased in 41+ weeks of gestation. Maternal age, parity, gravidity and mode of delivery did not affect the expression of VEGF. Fetal (birth weight, length, head circumference and abdominal circumference) and placental parameters (placental weight, volume, surface area and number of cotyledons) did not change with the VEGF level. Hemoglobin level less than 11 g/dL and male fetuses showed an increase in the VEGF level. The expression level was decreased in the abnormal umbilical cord and membranous attachment. The VEGF level was increased significantly in all the complicated pregnancies.

Conclusion: The VEGF expression was stabilized in the third trimester of pregnancy. The expression was increased in 41+ weeks due to placental insufficiency. The VEGF did not show a relationship with fetal and placental growth measures. Therefore, we can say that VEGF was not associated with growth, but its stabilized expression was important in maintaining pregnancy. Hence, the VEGF level at term cannot be useful in predicting fetal growth parameters. The VEGF level was reduced in the abnormal umbilical cord and membranous attachment of placentae. The reduced VEGF level may cause morphological malformations of the placenta. Also, the VEGF was increased in all the complicated pregnancies, significantly in PE and IUGR. Therefore, VEGF alone may not be beneficial in predicting a single disease.

Combinations with other markers will be beneficial in differentiating multiple complications.

Keywords: Blood vessels; Fetal development; Immunohistochemistry; Placenta; Pregnancy; Vascular endothelial growth factor.

1. INTRODUCTION

1.1 Background

The development of the fetus depends entirely on the structure, function and growth of the placenta.[1] The human placenta is haemochorial, where the mother's blood meets the chorion without rejection.[1] It is very important for the growth and development of the fetus in the womb and is a mirror of prenatal fetal development. Structurally it consists of villi, forms the functional components of the placenta, and consists of a layer of cytotrophoblast (CT), syncytiotrophoblast (STB) and a core of mesoderm with blood vessels (BV). These villi form the placental barrier and transfer nutrients from mother to fetus and waste products from fetus to mother. It also acts as a barrier to pathogens and the maternal immune system. The cells of these villi function as an endocrine gland and synthesize many growth factors, hormones and other bioactive products which maintain pregnancy.[1,2] To execute these functions, it requires normal placentation, which is marked by the development of the rich vascular network, the proliferation of trophoblast (TB), invasion of the endometrium and vasculogenesis, branching angiogenesis in the first trimester and nonbranching angiogenesis in second and thirst trimester in the placenta and endometrium.[3] Placentation is a complex process where conceptus survival requires an elaborate connection with the maternal vessels, a novel form of vasculogenesis. To achieve this, the placental unique epithelial cells, CT, acquire tumour-like properties; invade the interstitium of the decidua and inner third of myometrium (interstitial invasion), to attach conceptus to the uterine wall.[4] During the invasion, the mass of these cells recanalize the uterine arteries (endovascular invasion), increase the diameter and decrease the resistance of vessels. In this manner, arterioles are remoulded into

amalgamated vessels consisting of fetal and maternal cells which divert the uterine blood to the placenta to meet the requirements of the developing fetus.[4,5] Once the lumina reform, uteroplacental exchange occurs,[6] which determines the healthy viable outcome of the fetus. Reduced or increased proliferation leads to macrosomic or fetal growth restriction.[7] The surface area which is required for nutrient transfer depends on this. This differentiation is controlled by complementary actions of factors such as matrix metalloproteinase-9, vascular endothelial cadherin, vascular cell adhesion molecule 1, several integrins, which stimulates stem cell differentiation, proliferation and invasion.[6] Some of the physiological parameters such as oxygen tension, growth factors, cytokines, transcription factors, chemokines, and protein fragments regulate vessel growth and development.[8] Among these, angiogenic growth factors are the key regulators of vessels of the placenta. The vasculogenesis; formation of first capillaries within villi begins on the 21st day of post-conception by the appearance of macrophages which secretes angiogenic growth factors,[9] which stimulates the hemangioblast cells, which are precursors of endothelial cells derived from mesenchyme arrange like long cords within the villi and forms the first vessel.[9] This is followed by branching angiogenesis (starts from day 32 to week 25), the number of vessels will increase rather than the type of vessels. In this stage, existing capillaries start sprouting and branching and form a network of capillaries inside the villi.[9-11] After 26 weeks of gestation, nonbranching angiogenesis starts and continues till term.[12] Most of the pregnancy complications are closely linked to the development of vessels in the placenta, which is reflected through placental weight and fetal growth.[13-16] The vascular endothelial growth factor family proteins are the one who regulates placental vascularization throughout pregnancy.[16-18]

Vascular endothelial growth factor (VEGF):

VEGF is first identified in bovine pituitary follicular cells by Napoleone Ferrara and his colleagues at Genentech in 1989.[19] An only angiogenic factor having both vascular permeability and endothelial mitogenic property.[20] It is a dominant angiogenic growth factor and function as a principal regulator of vessel development. Involved in the development of both pathological and physiological angiogenesis.[20] VEGF is a dimer of 46kDa.[21] It is strongly stimulated by hypoxia through hypoxia-inducible factor-1 (HIF-1).[22,23] There are different types of VEGF namely; VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PlGF).[24-27] Among these VEGF-A or commonly called as VEGF is prominently present in the placenta.[28] The VEGF-A exists in six splice forms, having 121, 145, 165, 183, 189 and 206 amino acids (VEGF-A121, VEGF-A145, VEGF-A165, VEGF-A183, VEGF-A189 and VEGF-A206).[29] VEGF-A165 form the chief source of VEGF family proteins secreted in the placenta.[28] VEGF binds to 2 tyrosine kinase receptors; vascular endothelial growth factor receptor-1 [VEGFR-1 or fms like tyrosine kinase-1 (Flt-1)][30] and vascular endothelial growth factor receptor-2 [VEGFR-2 or kinase domain receptor (KDR)][31] which are present in TB and endothelial cells.[26] It also acts through Neuropilin-1 (NRP-1) and Neuropilin-2 (NRP-2).[29] Through these receptors, VEGF stimulates endothelial cells to form blood vessels.[32] To support this, gene knockout studies have identified the significance of VEGF proteins and receptors in vasculogenesis and angiogenesis and the mice die due to abnormal endothelial cell growth and blood vessel formation.[33,34] An organized angiogenesis is required for nutrient transfer and fetal development.

VEGF expression is upregulated by hypoxia, supporting the hypothesis that the placenta develops under a hypoxic condition that stimulates VEGF and helps in placental development.[35] Less oxygen stimulates VEGF expression via HIF-1, which interacts with the hypoxic response element (HRE) in the VEGF gene.[36,37] The increased VEGF stimulates the differentiation and proliferation of CT,[8] which helps in invasion and remodelling of the spiral artery.[4,5] Thereby VEGF helps in the placentation and initiates uteroplacental circulation. Failure in VEGF and VEGF receptor (VEGFR) interaction observed in pre-eclampsia (PE), suggest that in early pregnancy VEGF-VEGFR interactions are essential in the differentiation and survival of CT, which invades the uterine wall and channelizes maternal blood to the placenta.[6] VEGF helps in the formation of first blood vessel i.e. vasculogenesis and branching angiogenesis (formation of new vessel from the existing vessel) till mid-gestation, where its expressions are more secreted by CT cells.[38,39] After mid-gestation it helps in nonbranching angiogenesis till term with PlGF.[12]

VEGF regulates apoptosis of endothelial cells through the phosphatidylinositol 3-kinase (PI3K) pathway and stimulates proliferation, thereby helping in vasculogenesis in endometrium and placenta.[6,32,33] VEGF also helps in the secretion of nitric oxide (NO) by stimulating NO synthase, which is present in STB[40,41] and endothelial cells.[42] NO is a vasodilator that reduces the peripheral resistance of maternal blood vessels[43] and increases the cardiac output and plasma volume of the mother,[44] which is positively linked with fetal growth.[45] The VEGF-A also improves the permeability by phosphorylation of vascular endothelial (VE)-cadherin at Tyr-685 and Tyr-731.[46] This increases paracellular clefts allowing macromolecules 76kDa dextrans; this function of VEGF is assisted by PlGF and VEGF-A165b. The interplay of VEGF-A165 molecules with PlGF is essential in maintaining the vessel junctions

and for viable outcome in normal and complicated pregnancies.[47] All these functions of the VEGF is correlated with the growth in early pregnancy.[48] We, therefore, presumed that VEGF stimulates the growth of the placenta by increasing branching and non-branching angiogenesis thereby increasing surface area and vascular permeability for nutrition transport which is reflected through placental growth which in turn increases the fetal growth. Therefore, by studying placental and fetal parameters association with the VEGF level will explain its role in growth.

A marker that reflects placental function and adverse outcomes of pregnancies is in demand for research.[49] Many studies tried identifying VEGF as a marker of PE and intrauterine growth restriction (IUGR) pregnancies. However, the results have varied; some studies show increased and other show decreased expression of VEGF.[50-53] The placenta is a source of markers that will explain the mechanism behind the complications. A single marker alone may not predict the outcomes. Clinical features and fetal, placental and maternal parameters association with these markers may be helpful[54] because the normal placentae have different phenotypes and vary in pathology and molecular levels.[55]

1.2 Literature review

Successful pregnancy depends on the implantation and formation of fetal and maternal blood vessels to supply the increasing demand of nutrition for developing embryo.[56] VEGF-A is an angiogenic growth factor, induced by hypoxia, contributes to endothelial growth, differentiation, function and vascular permeability. Imbalance of pro- and anti-angiogenic factors can cause endothelial impairment. This will reduce uteroplacental blood flow and can cause fetal hypoxia and death.[57-59] The growth factors which stimulate and maintain vascular growth and function have developmental and clinical significance.[27] Many researchers have studied VEGF-A165 in the placenta and the serum/plasma level of mothers in normal and complicated pregnancies but the results are varied. The origin of VEGF is still unknown.

VEGF expression in human placenta and animals:

Sharkey AM et al first showed VEGF in the placenta by reverse transcription-polymerase chain reaction (RT - PCR) and in-situ hybridization method in the first trimester and term placenta. They found 189, 165, 145 and 121 isoforms of VEGF in the first trimester and term placenta. The VEGF messenger ribonucleic acid (mRNA) expression was found in STB, CT and Hofbauer cells (HC) of mesoderm in the placenta of the first trimester. Decidua of the first trimester uterus also showed the presence of VEGF. Strong expression of VEGF was also observed in the glandular epithelium and the uteroplacental junction adjacent to Nitabuch's stria. In term placenta, the VEGF mRNA was observed in STB and HC. In the uterus, the expression was observed in extravillous trophoblast (EVT). Amnion and chorion also expressed VEGF. The study concluded that the VEGF by EVT in decidua may be due

to the presence of macrophages in between the EVT cells. They also observed strong VEGF stain in the isolated mesenchymal cells within the villi, suggesting that VEGF expression within each villous was regulated by local factors. This study also showed low VEGF expression in the first trimester with increased expression at term. The complex expression of VEGF was involved in angiogenesis on both fetal and maternal parts of the placenta. This study concluded that the prime source of VEGF was from macrophages.[60]

Demir R et al studied VEGF and its receptor in villi of early pregnancy placenta (22^{nd}- 66^{th} day of post-conception, n=35). The VEGF expression was found moderately in CT, intense staining in HC and mesenchymal cells. The VEGF secreted from these cells recruits and helps in the formation of first angiogenic cells. In the stroma of villi, a significantly increased immunoreactivity was observed as pregnancy progressed. The expression was increased significantly between 24 and 34 days. Flt-1 and KDR were found on CT, haemangiogenic cords, stromal cells and endothelium. These receptors expression showed that they maintain placental vessel development in a paracrine and autocrine way through interactions with their ligands. This study concluded that the VEGF in CT cells stimulated vasculogenesis and the angiogenesis by stromal cells and HC together with TB cells.[61]

Vuorela et al identified the presence of VEGF, VEGF-B,VEGF-C and PlGF mRNAs in the term placenta (n=5). In situ hybridization analyses showed that the mesenchymal cells within the chorionic plate expressed VEGF mRNA. Immunohistochemical results showed protein expression in villous blood vessels and stroma. Hence, the cells of chorionic plate secrete VEGF, which diffuses through the

villi and acts on the endothelium of vessels of villi in paracrine mode, while the PlGF mRNA expression was observed in TB and protein in vascular endothelium.[27]

In one of the studies, Cooper JC et al studied VEGF and its receptor in human placenta and decidua by immunohistochemistry. The VEGF was found in HC of the placenta and decidual cells, glandular epithelium and EVT in the uterus of the first trimester (n=3). In term placenta (n=8) of cesarean section, expression of VEGF was found in STB, EVT and the extracellular material. Amnion did not show VEGF expression. The flt-1 receptor was found on endothelial cells in the first trimester and on EVT and HC in the term placenta. The expression of VEGF by HC can act on endothelial cells for controlling proliferation and permeability. The flt-1 expression regulates endothelial function by autocrine mode. This study concluded that these factors may be involved in the migration and differentiation of EVT until term.[62]

Zhou Y et al in an in-vitro and in-vivo (n= 19) study demonstrated VEGF expression in chorionic villi cells and fetal vessels in the villous stroma in the first trimester. The CT of the proximal cell column did not stain for anti-VEGF but the distal cell column showed increased immunoreaction. The CT in the superficial uterine wall and cells of the placenta, which occupied the lumen of uterine vessels, showed intense staining with anti-VEGF. The VEGF localization and intensity of staining did not change from first to the second trimester. They also observed anti-VEGF staining in stromal cells, BV in the villous mesenchyme and CT of term placenta. Their in-vivo assay indicates that VEGF expression regulates the differentiation and invasion of CT that are essential to early placental growth. This research revealed that members of VEGF family control survival of CT.[8]

Geva E et al studied the VEGF-A, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) and their role in vasculogenesis and angiogenesis in a first (n=7), second (n=7) and third trimester (n=10) placentae. They found a linear increased level of VEGF and Ang-1 mRNA in the first and third trimester, while Ang-2 mRNA expression was increased more than the VEGF and Ang-1 and decreased in the third trimester. VEGF mRNA was localized to CT, STB, stromal macrophages and in perivascular cells of first, second and third trimester placentae. But in PE (n=5), VEGF was found mainly in STB and fibrous stroma. This study found differential cellular expression of VEGF in PE and normal. The mRNA results were consistent with the protein expression. They concluded that these factors were important in placental vascular biology (vasculogenesis and angiogenesis) and remodelling in an autocrine or paracrine pathway.[56]

Ghosh D et al studied VEGF and PlGF in rhesus monkey at primary implantation site during lacunar (n=6), villous (n=9) and villous placenta (n=6) stages of early gestation. In the lacunar stage, CT lining the extraembryonic cavity and in TB, the trophoblastic plate showed VEGF. But the PlGF expression was less in these cells. With the development, VEGF and PlGF expressions were observed in CT, cells of anchoring villi in the villous stage but EVT expressed at a lower level. Expression of both the factor was also observed in amnion, chorion and stromal cells of villi.[63] These expressions were concordant with the mRNA expression. All the epithelial cells expressed VEGF but PlGF was found only in plaque epithelium in the lacunar stage. With the development, VEGF and PlGF in plaque cell decreased. But the VEGF was increased in glandular epithelium and PlGF was at a low level. Expression was less observed in stromal decidual cells, but in the villous stage, the expression

was increased. Stage dependent actions of these growth factors during implantation was important in establishing placental sufficiency.[63]

In one of the studies, natural killer (NK) cells from decidua of the first trimester expressed angiogenic growth factors.[64] These were the main sources of angiogenic growth factors at the maternal and fetal junction and helped for vascular remodelling.[65] VEGF mediated angiogenesis played a key role in the pathogenesis of defective placentation.[66] An in-vitro study revealed that VEGF/PlGF stimulated endothelial cells and monocytes to release coagulation tissue factor. The increased tissue factor production may cause pathological pregnancy complications like thrombosis.[67,68]

An in-vivo model study also showed the ability of VEGF to promote angiogenesis and a role as inducer of microvascular permeability.[69] This induced the extracellular matrix formation which favoured endothelial cell migration and new blood vessel growth.[70] Another cell culture study showed increased endothelial cell permeability after adding VEGF-A165a and a reduction in permeability with VEGF-A165b. VEGF-A165b reduced the VEGF-A165a mediated cell permeability. Adding PlGF to endothelial cell culture did not affect the permeability mediated by VEGF-A165a. Adding of VEGF-A165a disrupted the VE-cadherin junctions and increased the paracellular clefts, but VEGF-A165b or PlGF did not affect the cell junctions. However, VEGF-A165b abolished the effect of VEGF-A165a mediated disruption of cell junctions. Co-incubation of VEGF-A165b and PlGF to culture reduced the effect of VEGF-A165b on VEGF-A165a. They also noticed the PlGF competes with VEGF-A165b to bind with VEGFR-1 and inhibits the VEGF-A165b binding to VEGFR-1. Thus, inhibition of VEGF-A165a mediated endothelial cell permeability by VEGF-

A165b may be through the actions of PlGF on VEGFR-1. So it is clear that PlGF rescues the functions of VEGF-A165a from the VEGF-A165b inhibition. The interplay of VEGF-A165 molecules with PlGF was important in maintaining the placental endothelial junctions (barrier) and in viable outcome in normal and complicated pregnancies.[71]

Reports also showed that the VEGF through antiapoptotic Ras/Raf/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (MEK) and PI3K/Akt signalling pathways reduces apoptosis of endothelial cells and stimulates proliferation of endothelium. When impaired, these regulatory pathways can lead to an increase in apoptosis and thereby the death of developing embryo. Therefore, antiapoptotic molecules can be activated for helping in angiogenesis and VEGF can be targeted for antiapoptotic therapy.[72]

In one of the studies, macrophages were isolated from the placental villi to see the coordinated expression of tumour necrosis factor α- (TNFα-) and VEGF mediated signalling components in early (9-12 weeks) and late pregnancy (38-40 weeks). There was a minimal expression of TNFα- in macrophages and all the cells expressed VEGF. They also observed increased production of these factors with gestational age and reduction of their antagonist's soluble tumour necrosis factor receptor-1 (sTNFR-1) and soluble vascular endothelial growth factor-1 (sVEGFR-1). The expression of these factors by macrophages of the placenta showed their dual immune and morphogenetic role in the placenta. This study showed that these receptors share common regulatory pathways.[73]

Evans Phylip et al studied VEGF in 60 non-pregnant women and 363 pregnant women between 41-91 days of gestation. The study showed increased VEGF level in

pregnant women (2.13 pg/l) compared to non-pregnant women (1.10 pg/l). The serum VEGF level was positively correlated with gestational age and negatively correlated with maternal height and weight. It also had a positive correlation with progesterone and human chorionic gonadotropin hormone (HCG). This study suggested that VEGF production could be influenced by HCG and progesterone would have a direct effect on it and had influenced placental function. The VEGF may help in adjusting maternal cardiovascular system in early pregnancy.[74]

Clark et al studied VEGF and flt-1 receptors throughout the pregnancy. In the first trimester (normal sample), the VEGF expression was observed in the glandular epithelium, decidua, BV of villi and TB. Flt-1 was in TB, decidua and endothelium of BV expressed KDR. In the second trimester (anencephaly placenta sample), the VEGF expression was observed in EVT and TB. Flt-1 was in EVT and KDR by endothelial cells. The staining was darker in CT than STB as in the first trimester. In the third trimester (normal sample), expression was observed in STB, CT, endothelial cells, EVT, HC and diffused staining within the villi. Flt-1 was seen in TB and EVT cells. This study showed that VEGF and its receptors expression changed throughout gestation depending on needs.[75]

Basu J et al studied the VEGF isomer 165b in human placentae of 1st, 2nd and 3rd trimester pregnancy by employing enzyme-linked immunosorbent assay (ELISA) method in normal pregnancy. They observed that expression of 165b was 158.11 pg/100mg in the 1st trimester, 437.09 pg/100mg in the 2nd trimester and 239.68 pg/100mg in the 3rd trimester. The expression level was not affected by the parity and ethnicity of the mother. In the first trimester, gestational age demonstrated a strong association with VEGF-165b and the expression reduced thereafter.[76]

They also observed a difference in the level of 165b in women, those who received misoprostol and those who did not. This study concludes that 165b helps in vascular development and its expression in the placenta during development may antagonize the functions of VEGF, which could lead to complications in pregnancy.[76]

Expression of VEGF in complicated pregnancies:

Farias PS et al induced rats for diabetes and studied their placentae for VEGF expression. They found the increased intensity of staining for VEGF in diabetic than the normal group. VEGF was found in TB giant cells, glycogen cells and in spongiotrophoblast cells in the junctional zone. In the labyrinth zone, strong intensity of staining was observed only in TB. In a semiquantitative analysis of stained cells, they found that glycogen and TB giant cells stained more for VEGF in the diabetic group. Spongiotrophoblast staining was similar in both groups.[77]

Meng Q et al studied 20 placentae of diabetic pregnancy. The VEGF expression was observed in the STB layer. The VEGF mRNA and protein decreased in diabetic placentae. They also observed structural changes in the villi of the placenta. They concluded that decreased VEGF affected the placental angiogenesis and structure thereby placental function.[78]

Pietro L et al studied VEGF and its receptor in hyperglycemic pregnant women. The VEGF expression was found in STB, endothelial cells of BV, smooth muscles of vessels and mesenchymal cells of villi of the placenta. In maternal tissue, VEGF expression was observed in EVT, endothelium and muscles of vessels. The expression of VEGF in these cells and its intensity varied among diabetic groups. STB showed VEGF in all groups. The VEGF was expressed in endothelium and muscle cells of villi in the normal, hyperglycemic and clinical diabetic groups. The

gestational diabetes mellitus (GDM) group did not show VEGF in endothelium and muscle cells. VEGF expression showed in maternal vessels and CT cells of decidua in normal, mild glycemic and clinical diabetic groups. But EVT staining was weak in intense GDM. VEGFR-1 staining was observed in GDM, clinical and normal but weakly in milder hyperglycemic and staining was observed in vessels of villi and TB. VEGFR-2 was present in all cases. STB cells stained intensely in all groups. The endothelium of large vessels and capillaries and mesenchymal cells in normal and hyperglycemic women also showed expression. In a clinical diabetic woman, villi showed similar results except for capillary endothelium. In GDM women, staining was observed only in STB. EVT showed expression in all groups especially more in milder hyperglycemic women. They observed a discrepancy in VEGF staining in all groups. They concluded that hypercapillarization in mild hyperglycemia due to downregulation of VEGFR-1 which stimulated VEGF/VEGFR-2 interaction lead to angiogenesis. These changes were compensatory, caused by hemodynamic changes associated with hyperglycemia.[79]

A similar study conducted by Bhattacharjee D et al showed fibrinoid substance deposition, villous immaturity and chorangiosis as common histopathological changes in hyperglycemia, GDM and overt diabetic placenta.

In the normal placenta (n=12), the VEGF expression was observed in STB. Moderate staining was also observed in CT, villi mesenchyme and vessel endothelium. Cells of capillary endothelium showed strong staining.

In hyperglycemia (n=13), moderate VEGF expression was observed in STB, CT, mesenchymal cells and strong expression was seen in the capillary endothelium. However, weak expression was observed in endothelium and smooth muscle cells of blood vessels.

In GDM (n=33), moderate staining was observed in STB while a weak expression was seen in CT. No other tissue of the placenta showed VEGF expression.

In overt diabetes (n=18), STB and capillary endothelium showed strong expression of VEGF, while CT and mesenchyme of villi showed moderate, and vascular smooth muscles showed weak staining. Vessel endothelium did not show expression of VEGF.

In overall observation, the VEGF expression showed the most deviation from the normal in GDM placentae. The expression was similar to normal in hyperglycemic and more altered in overt diabetes. They concluded that an imbalance in the VEGF expression may cause several histopathological malformations in diabetic placenta.[80]

In one of the studies of induced PE in rats, decreased expression of VEGF (51.47 ± 17.52 pg/ml) in the PE group was found compared to normal (108.55 ± 21.07 pg/ml). They also found increased micro RNA155 (miR155) in PE. They concluded that increased expression of miR155 may downregulate the expression of VEGF in PE.[81]

Lyall et al studied serum VEGF level in the umbilical vein and protein expression in the placenta of PE (n=9), IUGR (n=9) and PE with IUGR (n=7) pregnancies through ELISA and immunohistochemistry. They found a decreased VEGF level in PE pregnancy to 50 pg/ml. In four patients with PE with IUGR, VEGF levels were same and were not statistically significant. But in IUGR pregnancy, the VEGF level was 175.35 pg/ml and this result was not statistically significant. VEGF immunoreactivity was found in STB and stromal cells. The degree of staining was reduced in all cases compared to controls. This study suggested that reduced VEGF

leads to impaired vessel development and the source of VEGF may not be from the hypoxic environment.[82]

One of the studies by Akercan Fuat et al studied 10 PE placental biopsies by immunohistochemistry and compared the results with controls (n=10). The VEGF level was increased in PE cases than controls.[83]

Ahmad Shakil et al found elevated levels of VEGF in PE and IUGR cases compared to normal. They also observed increased soluble forms like tyrosine kinase-1 (sflt-1) levels in PE cases and observed that withdrawal of sflt-1 produced endothelial migration (in-vitro method). The sflt-1 level was increased by hypoxia. They concluded that sflt-1 is an antagonist to angiogenesis and increased level of it may be the reason for inhibiting angiogenesis in PE.[84]

Sundrani et al studied promoter CpG methylation of VEGF, KDR and flt-1 genes, found differential methylation in the promoter region in preterm PE, term PE and normotensive. Expression of VEGF, KDR and flt-1 gene levels was more in preterm than the other groups. Deoxyribonucleic acid (DNA) methylation was altered in all the genes in PE cases. This study showed that altered methylation may be the cause of PE. Altered DNA methylation has a role in angiogenesis of placenta and leads to adverse outcomes.[85]

In one of the studies of hypertensive disorders, the VEGF was observed in TB, endothelial cells, stromal cells and HC in PE with hemolysis, elevated liver enzymes, low platelet count (HELLP, n=20). In gestational hypertension (GHP, n=20) and PE (n=20), the placental components (vessels, HC, stromal cells) were reactive but only some tracts of TB showed a reaction. mRNA level of VEGF in PE was lower compared to control. PE with HELLP mRNA level was statistically lower and was

higher in hypertension compared to control. This study showed differential expression of VEGF that depended on the severity of the disease, which might have been due to hemodynamic changes that occurred in these diseases to compensate the uteroplacental blood flow.[86]

In one of the studies, expression of VEGF, PlGF and their receptors were studied in PE placenta (early-onset and late-onset PE). The VEGF and PlGF were found in STB, BV and also in mesenchymal cells. The flt-1 was also expressed by similar cells. They found a decreased level of these proteins in PE cases compared to normal. They found more structural impairments of the placenta in early-onset of PE than late-onset.[87]

A study showed predominant expression of isoform 121 in the placenta of PE, IUGR and normal cases. The level of VEGF isoform and total VEGF mRNA was more in PE and IUGR compared to control. Maternal free and bound VEGF was higher in PE and IUGR compared to control. They also found that sflt was more in PE and IUGR. The VEGF expression was observed in STB, CT and EVT.[88]

In another study, the VEGF was noticed in STB, CT cells, fetal and maternal endothelial cells and decidual cells. All the cases of normotensive and hypertensive groups showed endothelial expression. The endothelial expression was more compared to the hypertensive cases (n=15), but it was not statistically important. The STB cells showed expression in 93.3% of cases in the hypertensive group, but in the normotensive group, only 13.3% showed VEGF expression. The differences in the expression were statistically significant. The intensity of the staining of STB cells was strong in the hypertensive group and moderate in the normotensive group. The expression of VEGF in CT cells was 40.0%, in decidual cells was 40.0% and in maternal endothelial cells was 6.7% in the hypertensive group. In a normotensive

group, the expression of VEGF in CT cells was 33.3%, in decidual cells was 73.3% and maternal endothelial cells was 20.0%. In this study, VEGF in STB was significantly increased in the hypertensive group; this could be used as a biomarker of hypertensive pregnancy.[89]

Ali LE et al studied PlGF, VEGF and hypoxia-inducible factor-1α (HIF-1α) in severe PE (n=62), mild PE (n=102) and controls (n=101) by immunohistochemistry. The expression of PlGF was observed in 16% of severe PE, 8.8% of mild PE and 40.6% of control placentae. The intensity of expression of PlGF was lower in severe PE compared to mild and controls which was statistically significant. The VEGF was expressed in 32%, 17.6% and 14.9%, in the severe PE, mild PE and control placentae respectively. The expression of VEGF was more in severe PE which was statistically significant. HIF-1α expressed in 15%, 10.8% and 5.0% of the severe PE, mild PE and control placentae respectively. None of the placentae showed high intensity for HIF-1α. They concluded that these three factors were involved in the pathophysiology of PE.[90]

In another study, decreased serum VEGF expression was reported in pregnancy-induced hypertension (PIH, n=36) compared to normal pregnancy (n=36). The systolic and diastolic blood pressure was negatively correlated with the serum VEGF level. They also noticed significantly increased sflt-1 in PIH mothers compared to normal mothers. The systolic and diastolic blood pressure was positively correlated with the increased sflt-1. The increased sflt-1 will bind to the VEGF and reduce free functional VEGF. This was, may be the reason for a decrease in serum VEGF in PIH mothers. Histological examinations of these placentae showed increased syncytial knot formation, fibrinoid necrosis, CT cell proliferation, stromal fibrosis, hyalinization and calcification in PIH mothers. This study suggested that increased

systolic and diastolic blood pressure with altered pro- and anti-angiogenic growth factors will help in identifying PIH mothers in the early stage to refer them to a higher health care centre for better management of complications of PIH.[91]

An immunohistochemical study reported increased expression of VEGF in PE (n=20) placentae compared to normal (n=20). The VEGF expression was found in cells of blood vessels of villi in normal. In PE, VEGF was found in endothelial cells and also in HC. Differential cellular expression of VEGF was noted in this study. They also observed decreased placental and fetal weight in PE cases than normal. Calcification, infarction and velamentous cord insertion were more in PE placentae.[92]

Andraweera PH et al studied VEGF mRNA in the placentae of adverse pregnancy outcomes by RT-PCR. They observed reduced mRNA (53%) expression in PE (n= 18) placentae compared to uncomplicated pregnancies. In GHP (n= 15), it was reduced by 47%, in normotensive small for gestational age (SGA, n=13) pregnancies it was reduced by 42%, in spontaneous preterm birth (n=10) it was reduced by 58% compared to normal pregnancy (n=30). This study concluded that the common pathway behind these complications would help in future for screening and treatment of diseases.[93]

Some RT-PCR studies also showed decreased levels of VEGF mRNA in PE and the cellular expression of VEGF was found in endothelial cells of BV of villi.[94,95] But a study by Geva E et al showed increased VEGF mRNA in PE cases.[56]

The pathophysiology of PE was potentially linked to B–cell-mediated production of autoantibodies (AA). One of the studies showed the presence of AA for VEGF-A, VEGFR-1 and PlGF angiogenic growth factors in the placenta. They observed that in nulliparous, AA VEGFR-1 and AA PlGF and in multiparous, AA

VEGFR-1 were reduced significantly in PE compared to controls. In non-obese PE women, AA VEGFR-1 and AA PlGF and in obese with PE, levels of AA VEGF-A and AA VEGFR-1 were significantly reduced. In unadjusted analyses of the association of antibodies and PE showed that an association between AA VEGF-A and AA VEGFR - 1 with PE can be the predictor of PE. In nulliparous women, AA VEGFR-1 and AA PlGF were associated with PE. In multiparous, none of the AA was associated with PE. In non-obese, AA VEGFR-1 and AA PlGF were associated with PE. In obese women with PE, none of the AA was predictor of PE. In the group of PE and controls with overlapping ratios of sFlt/PlGF and AA, VEGF-A was associated with PE. This study suggested that antibodies had a protective role in pregnancy and might be the contributing factors for regulating antiangiogenesis.[96]

Placental VEGF promoter polymorphisms and its association with VEGF mRNA has been reported in PE and controlled pregnancy. The VEGF mRNA level was high in PE cases and was more in −634CC genotype compared to CG + GG genotypes of PE. This study found the −634CC genotype as a risk factor for PE. They did not find an association between VEGF mRNA and 1154G/A and −2549 I/D polymorphisms in both PE and controls.[97]

In a study, quantitative RT-PCR method revealed increased expression of miR-203 and reduced VEGF-A in PE (n=18) than the normal group (n=20). A negative association was found between miR-203 and VEGF-A. The downregulation of VEGF-A was, may be due to the upregulation of miR-203. The immunohistochemical staining of VEGF-A was found in villous TB along with weak staining in the endothelium of the placenta. The VEGF-A protein was down-regulated in PE compared to normal; this was, may be associated with the occurrence of PE.

The TB cell lines from the first trimester showed overexpression of miR-203, which reduced the proliferating capacity of cells and the inhibition of miR-203 showed increased proliferation. Transwell migration and invasion assay showed that miR-203 increased the capacity for invasion and TB migration. The miR-203 reduced the level of VEGF-A mRNA and protein. This study showed that miR-203 was involved in the occurrence of PE through decreasing VEGF-A which reduced the invasion, proliferation and migration of TB. The relationship between VEGF-A/miR- 203 was, may be the reason for onset/progression for PE that can be used as a biomarker or a pathway to develop a drug to treat PE.[98]

In one of the studies, 60 angiogenic factors (pro- and anti-angiogenic) were studied in mild PE (n=21) patients and normal (n=27) by the protein quantitative macroarray method. They found an increased concentration of 8 proteins, leukemia inhibitory factor, interleukin-6, interferon-gamma, hepatocyte growth factor, heparin-binding EGF-like growth factor, C-X-C motif chemokine-10, platelet-derived growth factor-BB and leptin, which are involved in the pathogenesis of PE. They also observed a decrease in VEGF, PlGF and follistatin level in the plasma of PE cases. The VEGF had a positive correlation with follistatin, so reduced follistatin level was may be due to reduced VEGF. This may be the new pathway for PE. This study identified some of the novel markers, which can be used as biomarkers for diagnosis of PE.[99]

In one of the studies, Ahmed MA et al have showed an elevated expression level of TNFα- and decreased level of VEGF mRNA. This study revealed these proteins as markers for PE.[8] Another study also showed an increase in VEGF mRNA in PE cases. In this study, the non-severe PE group showed a significant rise in the

VEGF compared to a severe group of PE where VEGF was decreased. This study concluded that VEGF had a major role in PE.[9] In another immunohistochemical study, decreased expression of VEGF in PE was reported and this study infered that early occurrence of PE was associated with impaired villous development.[100]

A study showed miR-152 and VEGF expression and a correlation between them in the placental tissue of rats. They found a rise in miR-152, VEGF mRNA and protein in PE cases. Increased miR-152 was positively correlated with VEGF. The in-vitro experiment showed that miR-152 promoted apoptosis of TB cells by increasing pro-apoptotic gene Bax and reducing anti-apoptotic gene B-cell lymphoma protein-2 at the same time.[101]

VEGF family proteins are important in angiogenesis. Imbalance in angiogenic proteins leads to PE, where sFlt-1 was increased.

A recent cell culture study had used magnetic beads coated with VEGF in PE. The functionalized magnetic beads with VEGF reduced the level of sFlt-1 and released PlGF to maintain angiogenesis. This study has provided a new therapeutical approach for PE.[102]

In one of the studies, Regnault TRH et al studied ovine placenta for VEGF, PlGF and its receptors in a IUGR model. Ligands and their receptors were identified in the ovine placenta for 55 and 90 days after coitus (dpc), as well as in maternal tissue. VEGF expression was increased with the gestational age. VEGF mRNA level was increased in hyperthermic ewes at 55 dpc compared to normothermic. At 90 dpc VEGF mRNA levels were not altered by hyperthermic treatment. The receptors mRNA increased throughout but in hypothermal receptors mRNA reduced. SVEGFR-

1 did not found in these tissues. They concluded that imbalance in these growth proteins and receptors lead to IUGR.[103]

In one of the studies, 42 placentae were studied for expression of VEGF in pathological conditions. Moderate to an intense level of VEGF mRNA and protein was identified in EVT and decidual cells. Expression was clear in hypoxic or ischemic changes like villous infarction, syncytiotrophoblastic knots, villous hypercapillarization and in achorionic plate with chorioamnionitis (CAM). The intensity of staining was more in tunica media of blood vessels of hypoxic/ischemic changed villi compared to normal. Less staining was observed in endothelium and mesenchyme and the expression was similar to those that were normal. But the mRNA expression was observed more in mesenchyme and less blood vessels of hypoxic or ischemic changed villi. The hypoxic or ischemic changed villi endothelial cells showed more expression of KDR. The intense expression of VEGF in blood vessels and KDR suggested paracrine regulation of cells of blood vessels by VEGF through KDR receptors to hypoxia or ischemia. Intense flt-1 mRNA was observed in the STB of hypoxic or ischemic villi but weak to moderate protein expression was observed in TB. Positive staining was also observed in infiltrated inflammatory cells in the chorion in CAM. These ligands may help with their migration. Immunoreactivity for KDR was absent here. In villitis, they did not find any positive reaction for any of these ligands and receptors in mononuclear cells, which are infiltrated in the villi, may be the phenomena of migration of inflammatory cells were different in CAM and villitis.[104]

Some studies found that VEGF and its receptors level decreased in the placenta of miscarriage and blighted ovum.[105]

Tseng JJ et al studied VEGF, PlGF and its receptors in the placenta of pregnancy complicated by placenta accreta. The immunohistochemical study of VEGF receptors in this study showed weak expression for VEGFR-2 in STB of cases than control. The VEGFR-2 in CT and EVT cells and VEGFR-1, VEGFR-3 in TB cells were not different in cases and controls. Western blot analyses showed expression in STB of 70% of samples. ELISA results revealed a higher level of VEGF (31.1G8.4 vs. 12.7G3.7 pg/ml) in accreta than normal. The sVEGFR-1 levels were lower in accreta compared to normal pregnancy (699.0G542.5 vs. 1573.4G241.1 pg/ml). They concluded that other microenvironmental factors were the reason for the reduction of VEGFR-2. Reduced VEGFR-2 probably may not be for placental accreta, it may be to balance the pro- and anti-invasive factors to regulate the invasion. The increased VEGF and reduced sVEGFR-2 suggested that they act on paracrine mode to stimulate neovascularization in the development of accreta.[106]

In another study, VEGF expression was studied in the placenta of accreta patients (n=23) and its correlation was compared with the miR-518b. This had a close relationship with placental development. The VEGF was increased significantly in the placenta of accreta cases compared to the control group. The VEGF mRNA and the miR-518b level were also recorded more in the accreta group. This study showed that there was a close relationship between increased VEGF and the occurrence of placenta accreta because VEGF helps in the invasion of TB during implantation, which was important in the occurrence of placenta accreta. This study concluded that increased expression of miR-518b increased the invasion of TB and caused placenta accreta and its expression was correlated with increased VEGF. This molecular mechanism can help in the development of therapies against accreta.[107]

Distribution of VEGF was studied in 22 nonimmunologic hydropsfetalis (HF) of 25-41 weeks of gestation and was compared with the 75 normal pregnancies. In both the groups, VEGF expression was observed in STB throughout the gestation. Stromal cells of villi also showed VEGF in the normal placenta of the first trimester. Its expression also continued in term pregnancy in the HF placenta. Stromal expression and STB expression in HF showed poor villous vascular development and failed to participate in the function of angiogenesis and vascular permeability.[108]

A study showed the consumption of aspartame, an artificial sweetener would increase the expression of VEGF. Ultrastructural observation showed the condensed nuclei of endothelial cells, changes in nuclei of STB and CT cells. This study suggested that consumption of artificial sweeteners would have an effect on VEGF.[109]

VEGF plays a major role in physiological and pathological angiogenesis. Recently drugs have been developed for anti-VEGF–A neutralizing antibody and multikinase inhibitors for lung, renal, colorectal cancers and glioblastoma patients.[110,111] Anti–VEGFR-1 or anti–VEGFR-2 neutralizing antibody, soluble VEGFR-3, VEGFR-1 or VEGFR-2 peptide vaccine therapy[112] and anti-PlGF antibody 2 neutralizing antibody[113,114] are developed and undergoing clinical trials.[115]

VEGF's role in the growth of placenta and fetus in early pregnancy in human and animals:

Some of the studies showed an association between early expression of VEGF with placental and fetal parameters. A study by Wheeler T et al showed VEGF concentration in maternal serum and its association with placental and fetal growth in early pregnancy (14 weeks gestation). The study recruited 655 participants; after the exclusion, 596 normal pregnant women were taken for the study. They observed an

increased VEGF concentration up to 20 weeks of gestation. The VEGF concentration of early pregnancy was positively correlated with placental volume at 16-20 weeks, placental and fetal weight at delivery. VEGF level was significantly higher in women carrying female fetuses than the male fetuses. The study showed that the positive relationship between VEGF serum concentration with the growth of placenta and fetus was, may be due to the influence of VEGF on NO production.[48]

Another study showed the relationship between VEGF and placental and endometrial vascularity in the pig. They observed increased VEGF mRNA expression with several blood vessels in the placenta and endometrium throughout the gestation. This was consistent with the immunohistochemical expression of VEGF from early to late gestation. The VEGF mRNA was positively correlated with fetal weight, placental weight and placental efficiency. This study reported that increased blood vessels at the placental endometrial interface resulted in an increased nutrient transfer between mother and fetus. They also demonstrated that increased VEGF-165 isoforms with fetal weight had the potent ability to increase vascular permeability.[116]

In another study, VEGF expression was studied in diabetic placentae and its relationship with the weight of the fetus. They found that a diabetic group with large gestational weight babies (4370 ± 250) placenta showed increased VEGF expression (1.2816 ± 0.87) with more placental weight (790 ± 130). In fitted multiple regression models, they observed that VEGF expression and placental weight had a positive correlation with the newborn weight. They also found mean blood glucose and HbA1C as explanatory variables revealing its relationship with fetal weight in the third trimester.[117]

VEGF and von Willebrand factor (Factor VIII) was studied by immunohistochemistry in a different part of the uterus and the placenta at different stages of gestation and correlated with the fetal development. At 80 and 105 days of gestation, these factor levels were higher. They did not see a difference between the side of the uterus, segments, and fetal sex and were identified in all the segments of the uterus. A positive correlation was observed between VEGF and fetal weight at 80 and 105 days of gestation. They concluded that the level of these factors upregulated till the middle third of pregnancy, therefore vascular development occured before this stage.[118]

Literature shows differential expression of the VEGF level in normal and complicated pregnancies. There are no studies so far solely explaining the relationship between VEGF expression and its association with placental, fetal and maternal parameters to explain its role in growth in later stages of pregnancy.

1.3 Justification:

VEGF is an angiogenic growth factor; has been studied for its role in early pregnancy in vessel development and implantation. But there is a lack of literature on its role in growth in later stages of pregnancy and also discrepancies about its expression at term. Studying relationships between VEGF level with placental and fetal parameters will explain its role in growth. This factor has been studied as a predictive marker for PE and IUGR pregnancies. However, the results varied. So a marker alone may not be beneficial in understanding the pathology. Therefore, there is a need to study its expression level and its relationship with growth parameters at term and factors influencing the VEGF expression, which will be beneficial in understanding the outcome of the complicated pregnancies.

1.4 OBJECTIVE

- To study the expression of VEGF in fetal growth by placental morphology and its immunohistochemistry.

2. MATERIALS AND METHODS

Source of data: Study was conducted in the Department of Anatomy, J. N. Medical College, KLE Academy of Higher Education & Research, Belagavi. Samples were collected from the Obstetrics and Gynaecology Unit, Dr Prabhakar Kore Charitable Hospital, Belagavi.

Ethical clearance: The study was approved by KAHER's Ethical Committee.

Consent form: Detailed information about the study was explained to the mothers and consent has been taken from all the mothers.

Inclusion criteria:

Live birth placenta of:

- Singleton birth
- 32 weeks and more gestation period
- All mothers without any systemic diseases

Exclusion criteria:

- HIV patients
- Hepatitis patients

Sample size:

The sample size was calculated as follows:

Formula:

$n = Z^2_{1-\alpha} \times SD^2 / d^2$

$Z_{1-\alpha}$= 1.96 with 95% confidence interval

SD = Standard deviation

d = Tolerable error

Variable	Sample size (literature)[48,119]	d (tolerable error)	Sample size	10% attrition
VEGF Mean	3.2	0.15	237.7353	261.5088
SD	1.18			
Weight of placenta Mean	516.89	4.4	347.3683	382.1051
SD	41.84			
Volume of placenta Mean	506.51	4.4	349.5302	384.4832
SD	41.97			
Surface area Mean	270.89	4	181.1797	199.2976
SD	27.47			
Weight of fetus Mean	2813.6	50	102.3325	112.5657
SD	258.06			
Head circumference Mean	35.1	0.3	72.13671	79.35038
SD	1.3			
Abdominal circumference Mean	33.7	0.3	138.2976	152.1274
SD	1.8			
Length (Crown-heel) Mean	49.4	0.4	201.9241	222.1165
SD	2.9			
Vessels/Villi Mean	5.9	0.3	344.2757	378.7032
SD	2.84			

For each variable, the sample size was calculated, the highest sample size attained was 349. With a 10% attrition factor, the sample size was 384. The final sample size was adjusted to 385.

Data collection:

The collection of placenta samples was started on 7th August 2017 and continued until we reached the sample size i.e. 385, which was completed on 30th November 2017. Placentae were collected immediately after the delivery. They were first observed for the attachment of the cord and fetal membranes. After that, membranes were trimmed from the attachment and the umbilical cord was cut 2 cm away from the attachment. Placental variables like weight, volume, surface area and the number of cotyledons were recorded.

After recording the placental parameters, a section from the centre of the placenta was taken. Sectioned tissue was washed thoroughly to remove the blood and then transferred into sterilized plastic containers containing 10% neutral buffered formalin for fixing. Bottles were then brought to the Anatomy Laboratory for further processing **(Fig. 1 and 2)**.

Fig. 1: Samples from 1 - 300

Fig. 2: Samples from 300 - 385

1. **Recording of placental variables:**

i. Measurement of the weight of the placenta (Fig. 3):[119]

- The collected placentae were washed.
- They were wiped to remove the excess mucous, fluid and maternal blood.
- The umbilical cord of the placentae was trimmed approximately 2 cm from the insertion.
- The fetal membranes were trimmed.
- The weighing machine was plugged into an electric outlet.
- On/off key was pressed to start.
- Readings of the scale were ensured to 0.00 gm before keeping the placenta.
- The placenta was placed on the weighing scale.
- Readings were measured with an accuracy of 10 gm.

Fig. 3: Weight of placenta

ii. Measurement of volume of the placenta (Fig. 4 and 5):[120]

Scherle's water displacement method was used for measuring the volume of the placenta.

- The collected placentae were washed.
- They were wiped to remove the excess mucous, fluid and maternal blood.
- The umbilical cord of the placentae was trimmed approximately 2 cm from the insertion.
- The fetal membranes were trimmed.
- Water was taken in a 2000 ml measuring jar.
- The initial level of water was measured.
- The placenta was submerged into the jar.
- Care was taken to submerge the placenta completely in water.
- Displacement of water was measured after the submerging of the placenta.
- The volume of the placenta was calculated by,[120]

The volume of placenta = Initial level of water - displaced water level

Fig. 4: Initial level of water

Fig. 5: Displaced water level

iii. Measurement of the surface area of the placenta (Fig. 6 and 7): [121]

- The collected placentae were washed.
- They were wiped to remove the excess mucous, fluid and maternal blood.
- The umbilical cord of the placentae was trimmed approximately 2 cm from the insertion.
- The fetal membranes were trimmed.
- The placenta was placed on a flat surface.
- Using a measuring tape, the largest and smallest length of the maternal surface was measured.
- These measurements were used to calculate the surface area as follows:[121]

Surface area= π x dl x ds /4 [121]

dl: largest diameter

ds: smallest diameter

Fig. 6: Measuring largest diameter

Fig. 7: Measuring smallest diameter

iv. **Attachment of cord:** Umbilical cord was observed for the following types of attachment on the fetal surface:

- Central **(Fig. 8)**
- Eccentric **(Fig. 9)**
- Marginal **(Fig. 10)**
- Velamentous **(Fig. 11)**

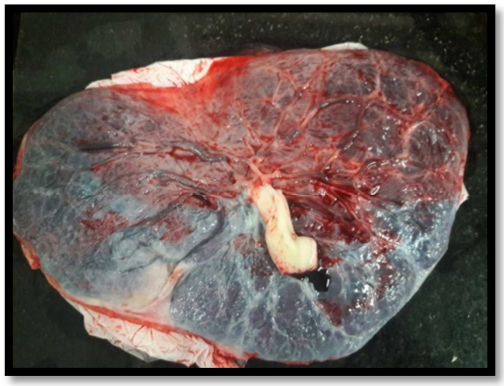

Fig. 8: Central cord attachment

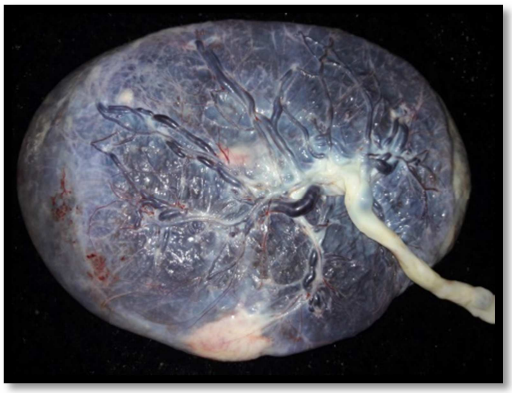

Fig. 9: Eccentric cord attachment

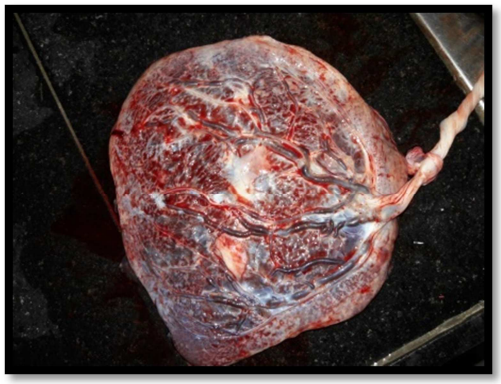

Fig. 10: Marginal cord attachment

Fig. 11: Velamentous cord attachment

v. **Membrane attachment:** Membranes of the placentae were observed for the following types of attachment:

- Membranous **(Fig. 12)**
- Circummarginate **(Fig. 13)**
- Circumvallate **(Fig. 14)**

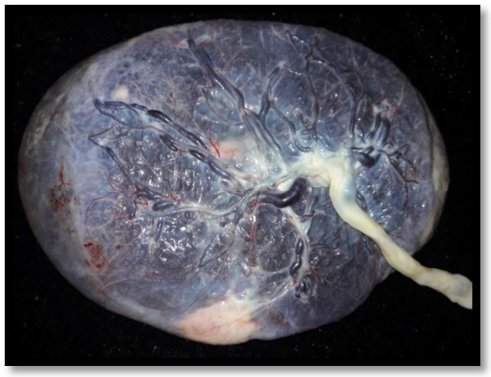

Fig. 12: Membranous attachment

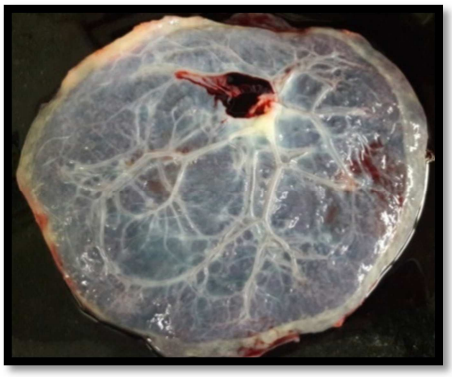

Fig. 13: Circummarginate attachment

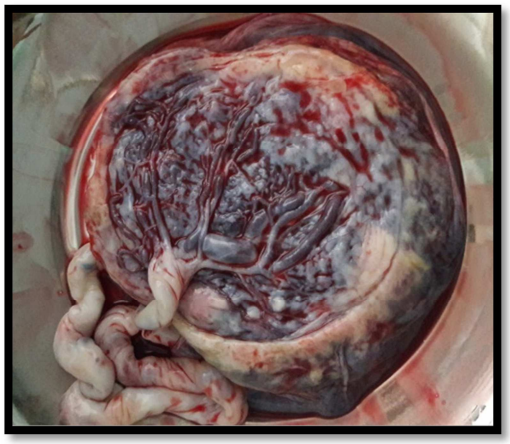

Fig. 14: Circumvallate attachment

vi. **Cotyledons count:** Cotyledons were counted on the maternal surface **(Fig. 15 and 16)**.

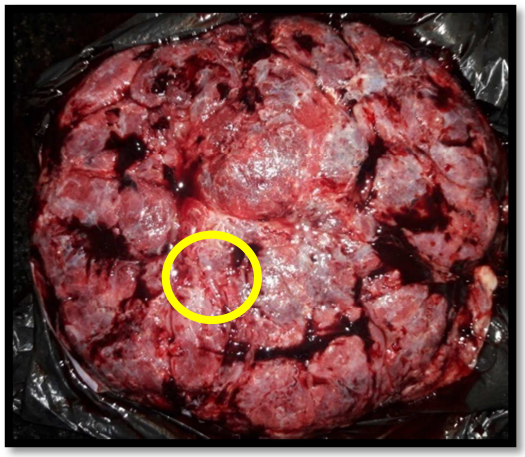

Fig. 15: Cotyledons of maternal surface (Circle indicates one cotyledon)

Fig. 16: Cotyledons counting

2. Recording maternal history:

On the next day of delivery, the history of a mother about the present and previous pregnancy and socio-demographic variables were recorded on predesigned proforma.

3. Methodology for fetal variables:

i. Recording gestational age of the newborn:

- Gestational age was recorded from the first day of the last menstrual period.

ii. Measurement of the birth weight of newborn:[122]

- Birth weight was estimated by the electronic gauging scale.
- The calibration of the weighing machine was done at the beginning of the study.

- On/off key was pressed to start the weighing machine. This will set the scale readout to zero.
- The newborn was set on the centre of the weighing scale and the weight of the baby was measured with an accuracy of 1 gm.

iii. Measurement of length (Crown-heel length) of the newborn:[122]

The length of the baby was measured by an infantometer.[122]

- The newborn was placed on an infantometer in the recumbent position facing feet towards the foot piece and head towards the fixed headpiece.
- The head was placed in the Frankfort horizontal plane.
- The head was secured by lightly cupping our palms over the ears of the baby for proper alignment.
- With one hand, the child's legs were aligned by putting mild pressure on knees.
- At the same time with another hand, foot piece was pushed towards the child's heel to rest firmly.
- The feet were flexed opposite against the acrylic foot piece.
- The newborn length was measured in centimetres with nearest to 0.1 cm

iv. Measurement of head circumference:[122]

- The child was asked to put over the shoulder in the supine position by a parent or guardian.
- A tape was wrapped around the forehead slightly above the eyebrows in front.

- It was above the ears on the side and over the occipital prominence on the back of the head.
- The tape was moved up and down over the back of the head to locate the maximum circumference.
- The tape was tightened around the head to compress the hair and underlying soft tissues.
- Head circumference was measured nearest to 0.1 cm.

v. Measurement of the abdominal circumference:[122]

- Measurement was done by using a measuring tape.
- The child was measured naked.
- The newborn was positioned in a supine position.
- The measuring tape was extended around the umbilical region.
- The tape was positioned in a horizontal plane to the floor.
- Tightening the insertion of tape around the umbilical region and circumference was measured.
- Abdominal circumference was measured in centimetre nearest to 0.1 cm.

A. Preparation of tissue blocks and slides:

The tissues were fixed in 10% neutral buffered formalin and were further processed as follows:

- **Dehydration:** The tissues were dehydrated in 70%, 80%, 95% alcohol, one hour each, followed by three changes of 100% alcohol, one hour each.
- **Clearing:** Two changes of xylene, one hour each for clearing.

- **Impregnation with paraffin wax:** The tissues were kept in two changes of paraffin for two hours each for impregnation.
- **Embedding:** The tissues were embedded in a paraffin block **(Fig. 17 and 18)**.
- **Sectioning:** Paraffin-embedded tissue blocks were sectioned at a 4 - 5 μm thickness on a microtome.
- **Fixing the tissue on slides:** The sections were floated in a 60°C hot water bath and were taken onto 3, aminopropyltriethoxysilane (APES) coated slides for immunohistochemistry and on gelatin-coated slides for hematoxylin and eosin (H and E) staining.

Fig. 17: Tissue block preparation

Fig. 18: Prepared tissue blocks

B. Immunohistochemical staining:

To study the VEGF, the slides were processed further for immunohistochemical staining.

Reagents used:

- **VEGF-A primary antibody**

Mouse monoclonal VEGF-A primary antibody was procured from Diagnostic BioSystems USA Pleasanton (catalogue # PDM165) **(Fig. 19)**.

- **MACH 2 Universal Horseradish peroxidase (HRP) (catalogue # M2U522H) – Polymer detection kit contained (Fig. 19):**
    - Peroxidase block
    - Protein block
    - 3', 3' Diaminobenzidine (DAB) chromogen and DAB substrate buffer.
- **Buffers:**
    - Tris ethylene diamine tetraacetic acid (EDTA) buffer (pH 9) was used as an antigen retrieval solution.
    - Tris buffer saline (TBS, pH 7.2-7.5) was used as a wash buffer.
- Xylene for clearing and dewaxing.
- 100% alcohol for dehydration.
- Distilled water for wash and for preparing buffers.
- Harris hematoxylin for counterstaining.
- Dibutyl phthalate xylene (DPX) for mounting.

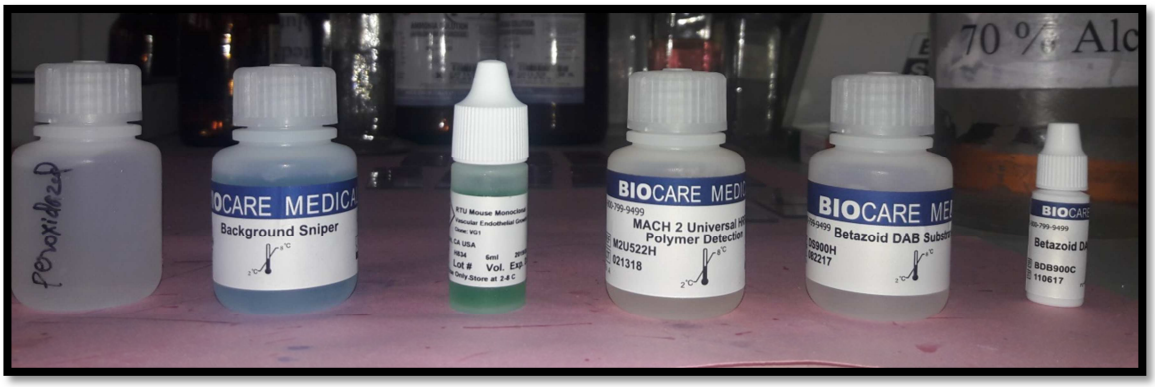

Fig. 19: Primary antibody (VEGF-A) and secondary kit

Preparation of antigen retrieval and wash buffers:

1. **Tris EDTA buffer (pH 9):**
    - EDTA – Disodium salt – 0.37 gm.
    - Tris buffer – 1.21 gm.
    - Distilled water – 1 lit.

2. **Tris buffer saline (TBS, pH 7.2-7.5):**
    - Tris buffer – 8.6 gm.
    - Sodium chloride – 9.6 gm.
    - Distilled water – 1 lit.

[PH was adjusted with 9% Hydrochloric acid (HCL) (9 ml HCL + 91 ml distilled water)].

The chemicals required for the preparation of buffers were procured from Nice Chemicals (P) Ltd, Kochi.

C. The methodology followed for immunohistochemical staining:

- **Fixing the sections:** The slides were incubated at 37^0 C in an incubator overnight.
- **Dewaxing:** The slides were kept on a hotplate at 63^0 C for one hour and then treated with two changes of xylene for ten minutes **(Fig. 20 and 21)**.
- **Dehydration:** Slides were treated with two changes of 100% alcohol for five minutes **(Fig. 21)**.
- **Wash:** Slides were washed with distilled water for five minutes.
- **Antigen retrieval:** Retrieval was done using a pressure cooker and induction stove. In a slide, basket slides were arranged and submerged in a pressure cooker containing one litre of antigen retrieval solution and heated on an induction stove for three whistles for half an hour. Then the cooker was cooled with lid for 15 minutes and without lid for 20 minutes before proceeding to the next step.
- **TBS wash:** The slides were washed with three changes of TBS for five minutes.
- **Peroxidase block:** The slides were treated with peroxidase block for ten minutes **(Fig. 22)**.
- **Protein block:** The tissues were rinsed with three changes of TBS for five minutes. Then the protein block solution was added and kept for 15 minutes.
- **Primary antibody incubation:** After 15 minutes, the excess of protein block solution was removed and the primary antibody was added onto the slides directly and incubated with the antibody for 45 minutes.

Then the slides were washed with three changes of TBS for five minutes.

- **Polymer – HRP:** The slides were treated with HRP for 30 minutes. Then the slides were washed with three changes of TBS for five minutes.
- **Chromogen:** After the TBS wash, a fresh DAB solution was prepared by adding 32 ul of DAB chromogen per 1.0 ml of DAB substrate buffer and the slides were incubated with DAB solution for five minutes.
- **Counterstaining:** The slides were washed with water and kept in Harris hematoxylin solution for 30 seconds. After this, the slides were rinsed in running tap water and kept in a hot water bath (48^0 - 50^0 C) for one minute. Then the slides were dried on a hotplate.
- **Mounting:** The slides were dipped in xylene and mounted with DPX.

Positive control tissue and negative control tissue and staining: Here kidney tissue was used as a positive control for the VEGF-A antibody. Reagent control was used as a negative control by skipping the primary antibody. Both the controls were run together with the sample tissue for every lot of stain.

Fig. 20: Dewaxing

Fig. 21: Dewaxing and clearing

Fig. 22: Immunohistochemical staining

D. Interpretation of immunohistochemical staining:

The stained slides were observed for,

- Localization
- Intensity

Localization of VEGF-A antibody staining in placenta tissue: VEGF-A antibody is a cytoplasmic and extracellular protein. Cytoplasmic staining of VEGF-A was found in the cells of the villi of the placenta **(Fig. 23 and 24)**. Depending on the expression, the intensity was measured from these cells in Image J software.

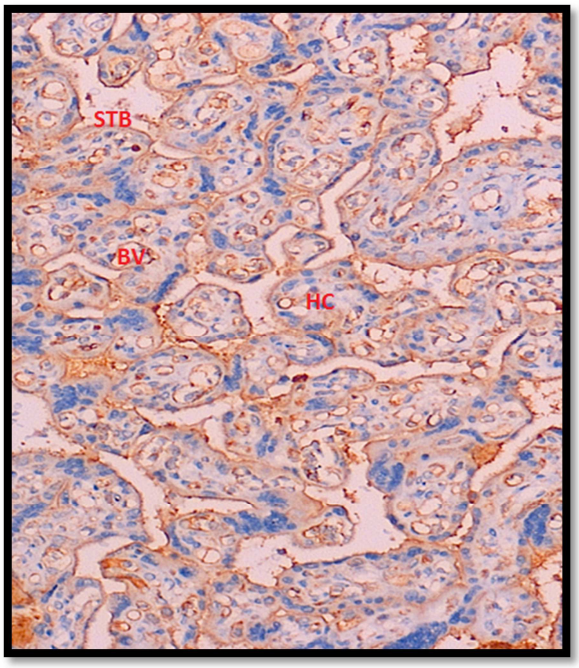

Fig 23: VEGF expression in syncytiotrophoblast cells (STB), Hofbauer cells (HC) and blood vessels (BV) of villi of placenta

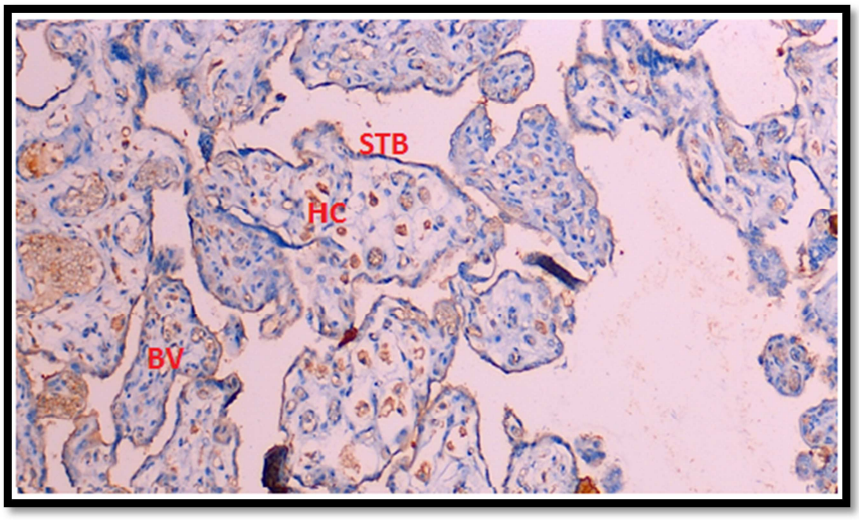

Fig. 24: VEGF expression in syncytiotrophoblast cells (STB), Hofbauer cells (HC) and blood vessels (BV) of villi of placenta

Intensity quantification using Image J software: In immunohistochemically stained slides, the stained area appears as varying degrees of colouration to the human eye, the darker regions holds more proteins but this area has lower intensity values, bringing about an opposite relationship between the measure of antigen and its

numerical worth. This will give a false result on the level of antigen. To overcome this a new technique can be adapted, i.e. 'reciprocal intensity'.[123]

The maximum intensity of a red, blue, green colour (RGB) is 250 in the white unstained area of images estimated by standard intensity function in the Image J software. The stained area will have an intensity of less than 250. Thereby reciprocal intensity is calculated by subtracting minimum intensity from the maximum. That corresponds to the amount of protein present.[123]

Steps followed:

1. Stained slides were observed under the research microscope and were photographed, numbered and saved.
2. Photographed slide pictures were opened in Fiji (Image J) software.
3. Uniformly sized circular draw tool (100 x 60) was drawn over the region of interest and the average intensity was calculated by measure tool under the menu 'Analyze'.
4. Then the mean intensity was deducted from the maximum intensity to get reciprocal intensity. Reciprocal intensity corresponds to the amount of protein present.
5. Reciprocal intensity is nothing but quantified VEGF in the tissue of the placenta.
6. The reciprocal intensity was measured for all 385 placental tissues.

A. **Methods followed for H and E staining:**

- The slides were deparaffinized for five minutes with two changes of xylene.

- Then the slides were kept in 100% alcohol, for two changes, five minutes each and 95%, 70% and 50% alcohols, respectively, for three minutes each.
- Water wash - five minutes.
- Hematoxylin - 45 seconds
- Bluing
- Dipping in eosin - 30 seconds
- Water wash
- Dehydration with absolute alcohol - one dip
- The slides were dried
- Cleared with xylene
- Mounted in DPX.

B. Methods followed for interpretation of H and E stained slides:

- The slides were focused under 40X.
- Here we counted the number of vessels in ten randomly selected terminal villi **(Fig. 25 and 26)** from a different region and an average of ten villi vessels was taken for the count.
- Observations were photographed.

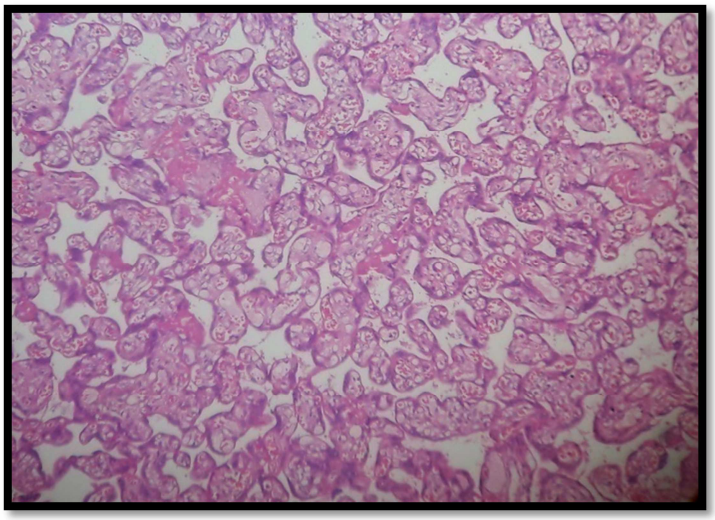

Fig. 25: Placenta H and E staining (10X)

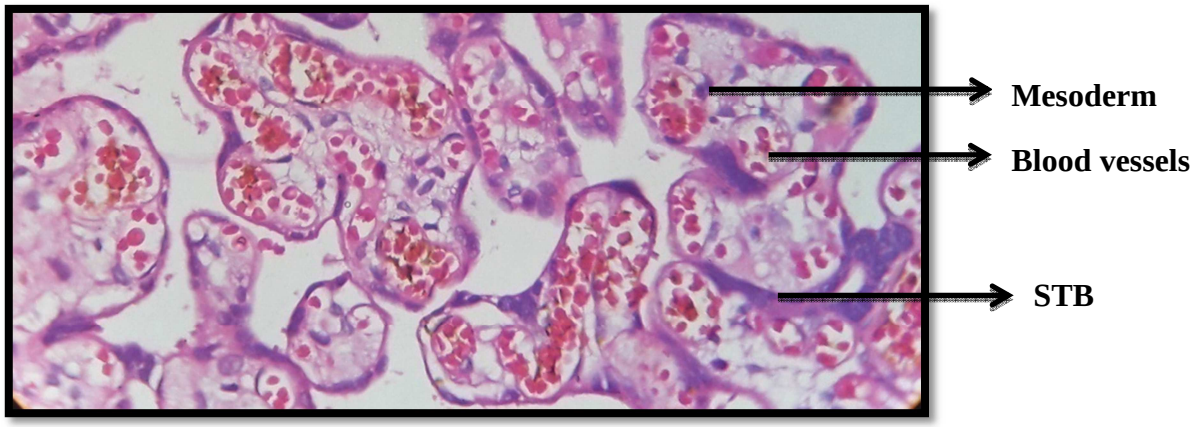

Fig. 26: H and E staining (40X) showing blood vessels in the terminal villi of placenta of normal

Definitions:

Gestational age: Counted from the first day of the last menstrual period to the present date. Recorded in weeks.[124]

Parity: Number of pregnancies reaching viable gestational age.[125]

Primipara: Women who have given birth to one viable child.[125]

Multipara: Women who have given birth to two or more viable children.[125]

Gravidity: Number of times the women were pregnant.[125]

Primigravida: Women who are pregnant for the first time.[125]

Multigravida: Women who have been pregnant for more than one time.[125]

PE: Occurs after 20 weeks of pregnancy with hypertension (140/90 mm Hg), protein in the urine, decreased in platelets count, altered liver and kidney function, fluid in lungs, new-onset of headaches and visual disturbances.[126]

GHP: Occurs after 20 weeks of gestation with pregnancy with hypertension (140/90 mm Hg), previously no blood pressure, no protein in the urine.[126]

Eclampsia (EC): Development of convulsions in preexisting PE.[127]

IUGR: Birth weight below the 10th percentile for the gestational age.[128]

GDM: GDM is characterized as any level of glucose intolerance with onset or first recognition during pregnancy.[129]

Anemia: Reduced hemoglobin level. Hemoglobin concentration <11 g/dL during the first trimester, <10.5 g/dL during the second trimester and <11 g/dL during the third trimester.[130]

Hypothyroid: Elevated thyroid stimulating hormone levels during pregnancy.[131]

Antepartum hemorrhage (APH): APH is characterized as bleeding from or into the genital tract, appears from 24+ weeks of pregnancy and before the birth of the baby.[132]

Premature rupture of membranes (PROM): PROM is the tearing of the fetal membranes before the beginning of labour.[133]

Lowbirth: Birth weight under 2500 g (up to and including 2499 g).[134]

Oligohydramnios: Reduced amniotic fluid (amniotic fluid index ≤5 cm, single deepest pocket <2 cm).[135]

Polyhydramnios: Excess of amniotic fluid (amniotic fluid index >18 cm, single deepest pocket >8 cm).[136,137]

Preterm birth: When birth occurs between 20 - 37 weeks of gestation.[138]

Congenital anomalies: Structural or functional defects that occur during intrauterine life.[139]

3. DATA ANALYSIS PLAN

1. First, the data was cleaned by checking for missing values and the outliers.
2. Mean and standard deviation was calculated for continuous variables and the percentage was calculated for categorical variables. Grouping of the continuous variables was also done.
3. The z-values were calculated for continuous variables and grouping of z-values of these variables was done.
4. To check the goodness of data, the chi-square test was applied by taking expected and observed frequencies. Using the midpoint of z-score, observed and expected frequencies, normal distribution curve was prepared to check whether it is normally distributed or not.
5. The categorical data were calculated for percentage and tabulated.
6. To check the role of VEGF in the growth of placenta and fetus, mean and SD of the intensity of STB, BV and HC cells were tabulated to each placental and fetal morphometrical variable and applied z-test to check the differences in VEGF level with the group of each variable.

Data inclusion criteria to check the role of growth of fetus:

- Placenta of 32 - 41 weeks of gestational age.

Data exclusion criteria:

- Mothers with hypertensive disorders of pregnancy (PE, EC, GHP).
- Mothers with oligohydramnios polyhydramnios, APH, PROM, GDM, placenta previa and placental abruption.

- Fetus with congenital anomalies.
- IUGR fetus.

7. Mean and SD of the VEGF expression in complicated pregnancies was taken (PE, EC, GHP, oligohydramnios, polyhydramnios, APH, PROM, GDM, a fetus with congenital anomalies and IUGR) and compared it with the VEGF expression of the normal placenta (37 - 41 weeks of gestation (70 cases), without any complication).

4. RESULTS

1. Percent distribution of maternal parameters:

Table 1: Percent distribution of maternal age at marriage

Z-score group of maternal age at marriage	Midpoint of z-score group of maternal age at marriage	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-3(-2)	-2.5	7	1.82	1.75
-2(-1)	-1.5	36	9.35	12.95
-1-0	-0.5	193	50.13	35.2
0-1	0.5	91	23.64	35.2
1-2	1.5	35	9.09	12.95
2-3	2.5	21	5.45	1.75
3+	3.5	2	0.52	0.09
Total		**385**	**100**	**100**
Mean= 20.42, SD= 3.26				

Graph 1: Percent distribution of maternal age at marriage

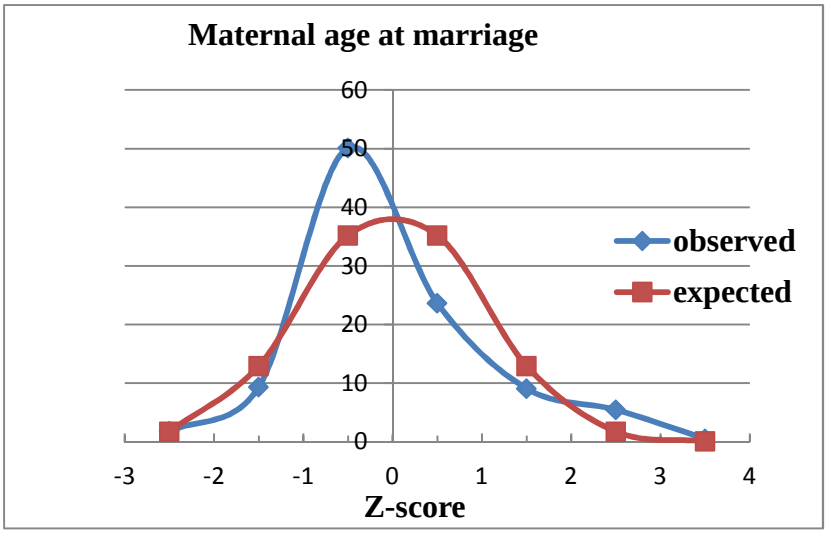

Table 1 and Graph 1 reveal that the percent distribution of maternal age at marriage was positively skewed with mean 20.42 and SD 3.26. In expected data, 50% of women were married above the average age, but in observed data, 38.70% were married above the average age, it was significant at p<0.001.

Table 2: Percent distribution of maternal age at first pregnancy

Z-score group of maternal age at first pregnancy	Midpoint of z- score group of maternal age at first pregnancy	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-_3(-2)	-2.5	1	0.26	1.75
-2(-1)	-1.5	57	14.81	12.95
-1-0	-0.5	190	49.35	35.2
0-1	0.5	88	22.86	35.2
1-2	1.5	31	8.05	12.95
2-3	2.5	15	3.9	1.75
3+	3.5	3	0.78	0.09
Total		**385**	**100**	**100**
Mean= 22.12, SD= 3.11				

Graph 2: Percent distribution of maternal age at first pregnancy

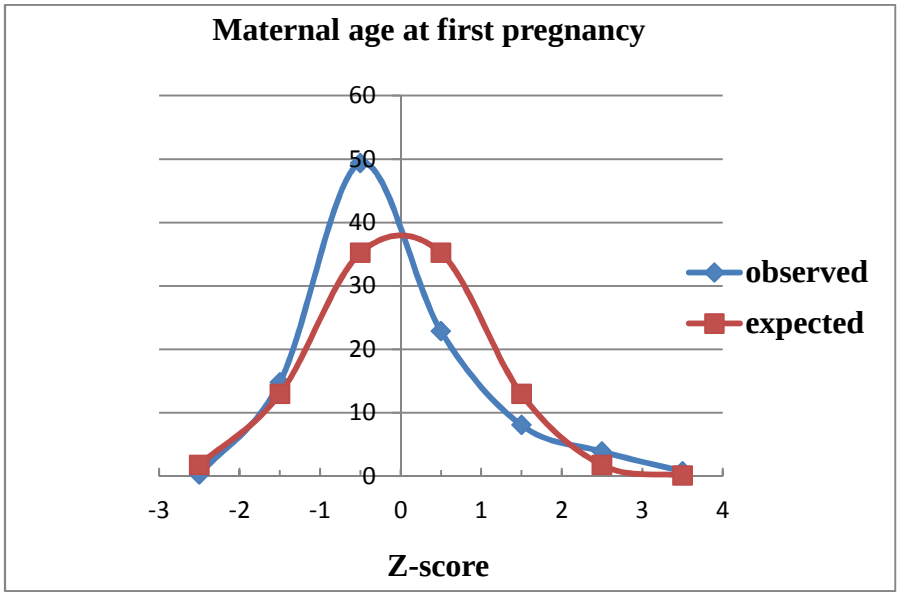

Table 2 and Graph 2 reveal that the percent distribution of age at first pregnancy of mother was positively skewed mean 22.12 and SD 3.11. In expected data, 50% of women were first pregnant at above the average age, but in observed data, 35.58% were first pregnant at above the average age, it was significant at $p<0.001$.

Table 3: Distribution of marital relations

Marital relations	Subjects (n)	Percentage (%)
Consanguineous	73	18.96
Non - consanguineous	312	81.04
Total	**385**	**100**

Table 3 shows 81.04% of marriages were non-consanguineous and 18.96% were consanguineous.

Table 4: Distribution of gravidity

Gravidity	Subjects (n)	Percentage %
Primigravida	159	41.30
Multigravida	226	58.70
Total	**385**	**100**

Table 4 shows the distribution of gravidity. In this study, 41.3% of mothers were primigravida and 58.70% were multigravida.

Table 5: Distribution of parity

Parity	Subjects (n)	Percentage %
Primipara	197	51.17
Multipara	188	48.83
Total	**385**	**100**

Table 5 depicts distribution of parity. In this study 51.17% were primipara mothers and 48.83% were multipara.

Table 6: Mode of delivery

Type of delivery	Subjects (n)	Percentage %
Normal vaginal	201	52.21
Cesarian section (C/S)	184	47.79
Total	**385**	**100**

Table 6 depicts 52.21% of women in this study group delivered normally and 47.79% of women delivered by C/S.

Table 7: Percent distribution of maternal hemoglobin level

Z-score of hemoglobin in g/dl	Midpoint of z-score of hemoglobin in g/dl	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
<-3	-3.5	2	0.52	0.09
-3-(-2)	-2.5	10	2.6	1.75
-2-(-1)	-1.5	42	10.91	12.95
-1-0	-0.5	134	34.81	35.2
0-1	0.5	135	35.06	35.2
1-2	1.5	57	14.81	12.95
2-3	2.5	5	1.3	1.75
Total		**385**	**100**	**100**
Mean= 11.08, SD= 1.31				

Graph 3: Percent distribution of maternal hemoglobin level

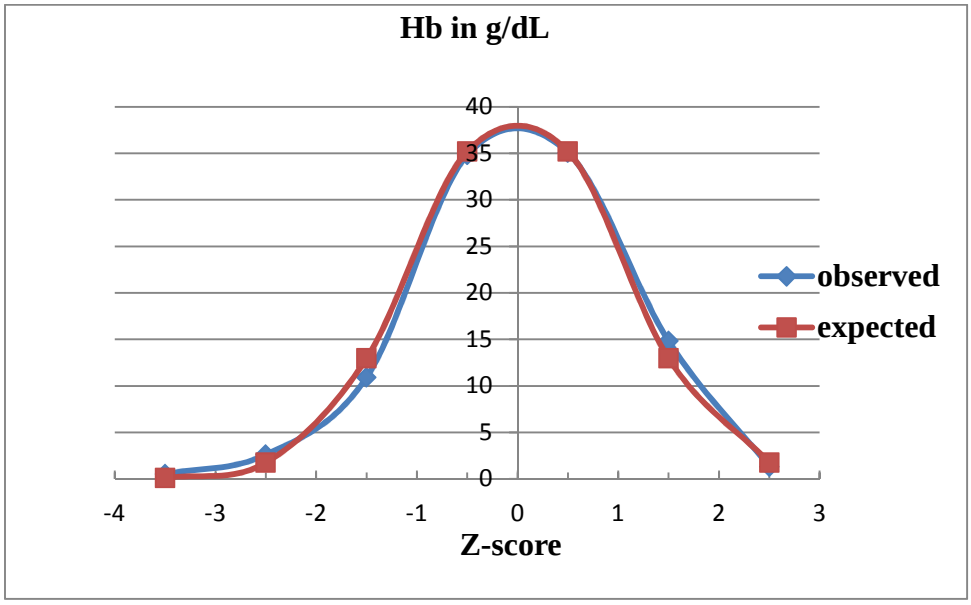

Table 7 and Graph 3 reveal that the percent distribution of maternal hemoglobin was normally distributed with mean 11.08 and SD 1.31g/dL. There were no differences between the expected and observed distributions.

2. Percent distribution of fetal parameters:

Table 8: Percent distribution of gestational age

Z-score group of gestational age	Midpoint of z-score group of gestational age	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
<-3	-3.5	2	0.52	0.09
-3-(-2)	-2.5	11	2.86	1.75
-2-(-1)	-1.5	30	7.79	12.95
-1-0	-0.5	81	21.04	35.2
0-1	0.5	168	43.64	35.2
1-2	1.5	93	24.16	12.95
Total		**385**	**100**	**100**
Mean=37.98, SD=2.01				

Graph 4: Percent distribution of gestational age

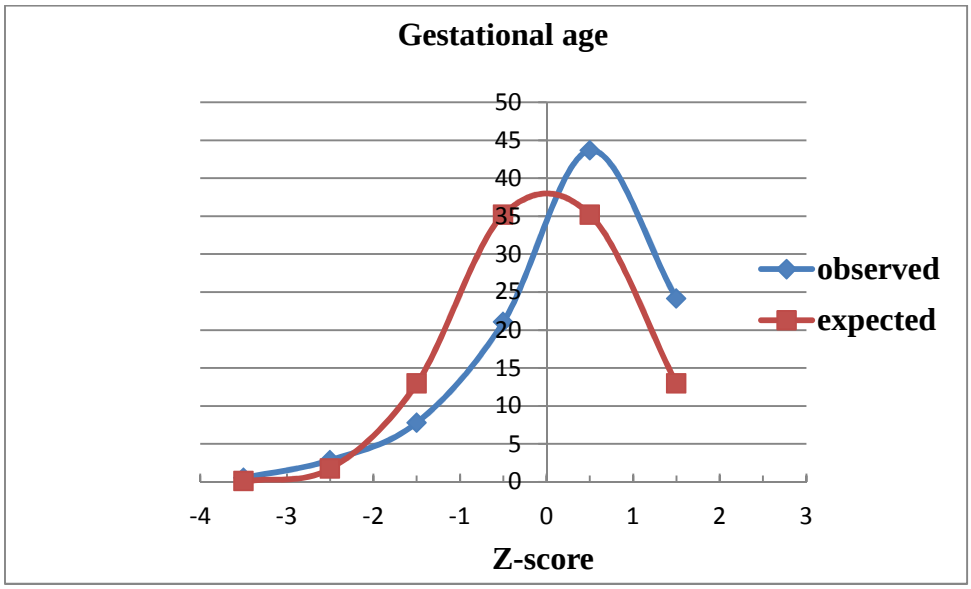

Table 8 and Graph 4 reveal that the percent distribution of gestational age was negatively skewed with mean 37.98 and SD 2.01 weeks. In expected data, 50% fetal gestational age was below the average age, but in observed data, 32.21% fetal gestational age was below the average gestational age, it was significant at $p<0.001$.

Table 9: Percent distribution of birth weight

Z-score group of birth weight	Midpoint of z- score group of birth weight	Subjects (n)	Frequency distribution	
			Observed (%)	Expected (%)
<-3	-3.5	4	1.04	0.09
-3-(-2)	-2.5	13	3.38	1.75
-2-(-1)	-1.5	42	10.91	12.95
-1-0	-0.5	114	29.61	35.2
0-1	0.5	162	42.08	35.2
1-2	1.5	45	11.69	12.95
2-3	2.5	5	1.3	1.75
Total		**385**	**100**	**100**
Mean=2658.71, SD=471.27				

Graph 5: Percent distribution of birth weight

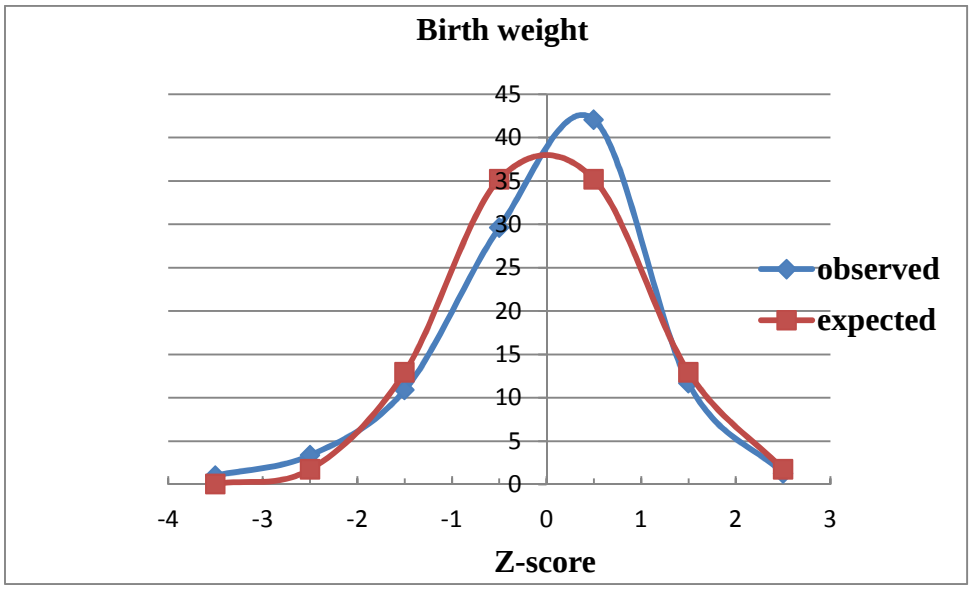

Table 9 and Graph 5 reveal that the percent distribution of birth weight was negatively skewed with mean 2658.71 and SD 471.27 gm. In expected data, 50% birth weight was below the average birth weight, but in observed data, 44.94% birth weight was below the average birth weight. The difference between them was not statistically significant.

Table 10: Percent distribution of fetal length

Z-score group fetal length	Midpoint of z-score group fetal length	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
<-3	-3.5	4	1.04	0.09
-3-(-2)	-2.5	15	3.9	1.75
-2-(-1)	-1.5	31	8.05	12.95
-1-0	-0.5	143	37.14	35.2
0-1	0.5	137	35.58	35.2
1-2	1.5	53	13.77	12.95
2-3	2.5	2	0.52	1.75
Total		**385**	**100**	**100**
Mean=48.02, SD= 3.61				

Graph 6: Percent distribution of fetal length

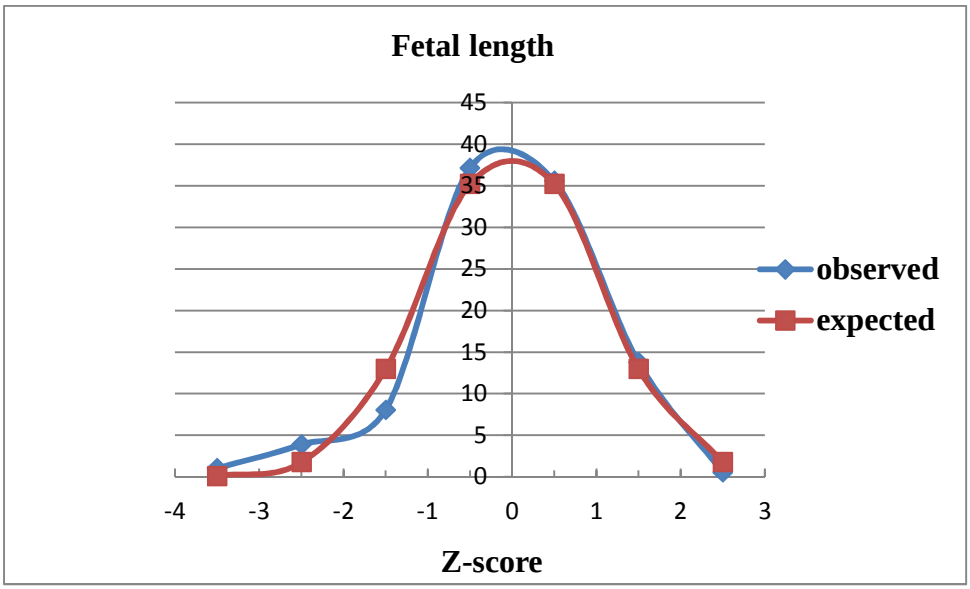

Table 10 and Graph 6 reveal that the percent distribution of fetal length was normally distributed with mean 48.02 and SD 3.61cm. There were no differences between the expected and observed distributions.

Table 11: Percent distribution of fetal head circumference

Z-score group fetal head circumference	Midpoint of z-score group fetal head circumference	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
<-3	-3.5	8	2.08	0.09
-3-(-2)	-2.5	11	2.86	1.75
-2-(-1)	-1.5	26	6.75	12.95
-1-0	-0.5	122	31.69	35.2
0-1	0.5	173	44.94	35.2
1-2	1.5	42	10.91	12.95
2-3	2.5	3	0.78	1.75
Total		**385**	**100**	**100**
Mean= 33.53, SD= 2.22				

Graph 7: Percent distribution of fetal head circumference

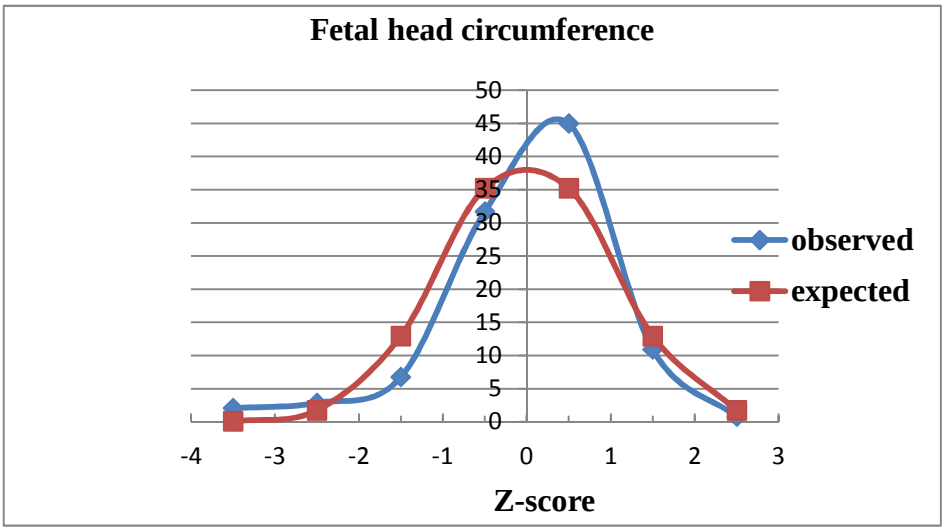

Table 11 and Graph 7 reveal that the percent distribution of fetal head circumference was negatively skewed with mean 33.53 and SD 2.22 cm. In expected data, 50% fetal head circumference was below the average head circumference, but in the observed data, 43.38% of babies fetal head circumference was below the average head circumference, it was significant at $p<0.05$.

Table 12: Percent distribution of fetal abdominal circumference

Z-score group of abdominal circumference	Midpoint of z-score group of abdominal circumference	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution(%)
<-3	-3.5	1	0.26	0.09
-3-(-2)	-2.5	6	1.56	1.75
-2-(-1)	-1.5	47	12.21	12.95
-1-0	-0.5	121	31.43	35.2
0-1	0.5	166	43.12	35.2
1-2	1.5	32	8.31	12.95
2-3	2.5	11	2.86	1.75
3+	3.5	1	0.26	0.09
Total		**385**	**100**	**100**
Mean=28.93, SD= 2.37				

Graph 8: Percent distribution of fetal abdominal circumference

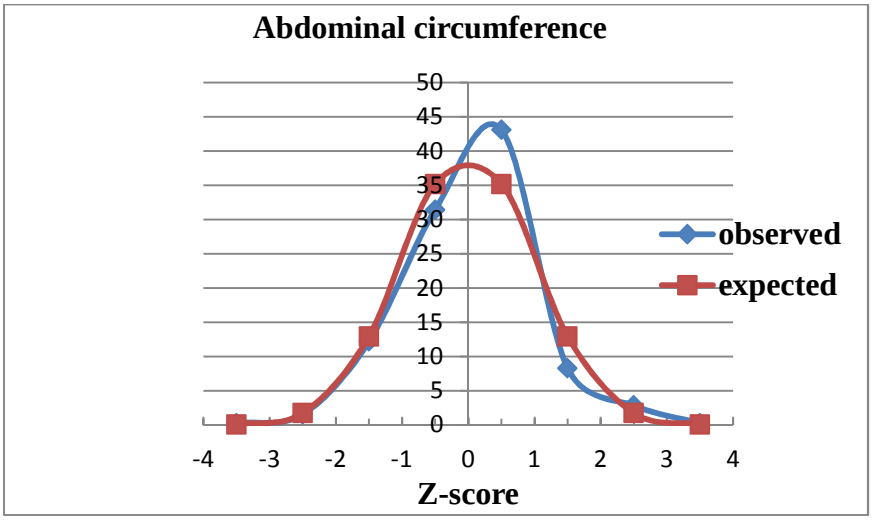

Table 12 and Graph 8 reveal that the percent distribution of fetal abdominal circumference was negatively skewed with mean 28.93 and SD 2.37 cm. In expected data, 50% fetal abdominal circumference was below the average abdominal circumference, but in observed data, 45.45% fetal abdominal circumference was below the average abdominal circumference. The difference between them was not statistically significant.

Table 13: Distribution of sex of the fetus

Sex of the fetus	Subjects (n)	%
Male	195	50.65
Female	190	49.35
Total	**385**	**100**

Table 13 shows the distribution of sex of the fetus. 50.65% of newborns were male and 49.35% were female.

3. Percent distribution of placental parameters:

Table 14: Percent distribution of the weight of the placenta

Z-score group of weight of placenta	Midpoint of z-score group of weight of placenta	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-3-(-2)	-2.5	6	1.56	1.75
-2-(-1)	-1.5	53	13.77	12.95
-1-0	-0.5	145	37.66	35.2
0-1	0.5	127	32.99	35.2
1-2	1.5	43	11.17	12.95
2-3	2.5	9	2.34	1.75
3+	3.5	2	0.52	0.09
Total		**385**	**100**	**100**
Mean=420.39, SD= 94.81				

Graph 9: Percent distribution of the weight of the placenta

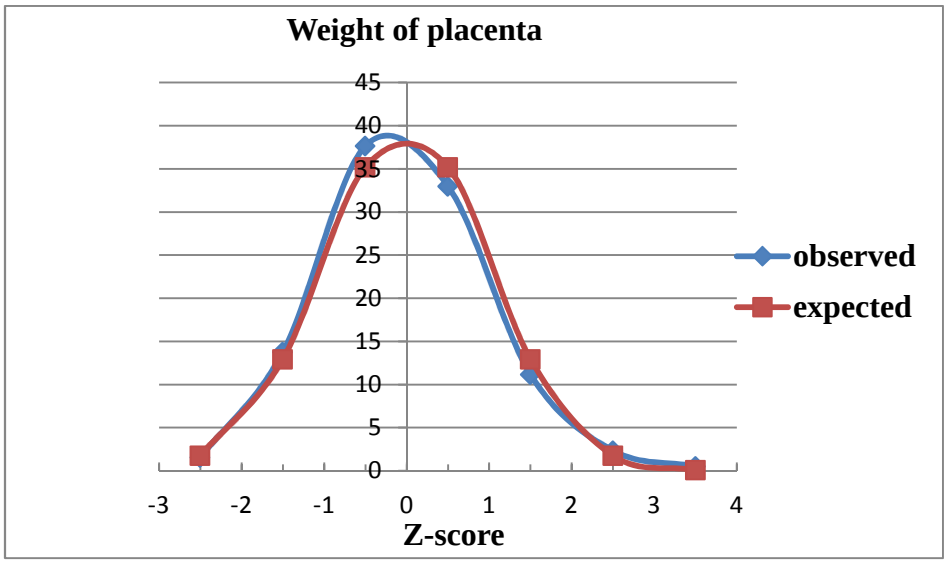

Table 14 and Graph 9 reveal that the percent distribution of the weight of the placenta was positively skewed with mean 420.39 and SD 94.81 gm. In expected data, 50% placenta weight was above the average placenta weight, but in observed data, 47.01% were above the average. The difference was not significant.

Table 15: Percent distribution of the volume of placenta

Z-score group of volume of placenta	Midpoint of z-score group of volume of placenta	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-3-(-2)	-2.5	6	1.56	1.75
-2-(-1)	-1.5	53	13.77	12.95
-1-0	-0.5	148	38.44	35.2
0-1	0.5	125	32.47	35.2
1-2	1.5	40	10.39	12.95
2-3	2.5	10	2.6	1.75
3+	3.5	3	0.78	0.09
Total		**385**	**100**	**100**
Mean= 415.86, SD= 95.52				

Graph 10: Percent distribution of the volume of placenta

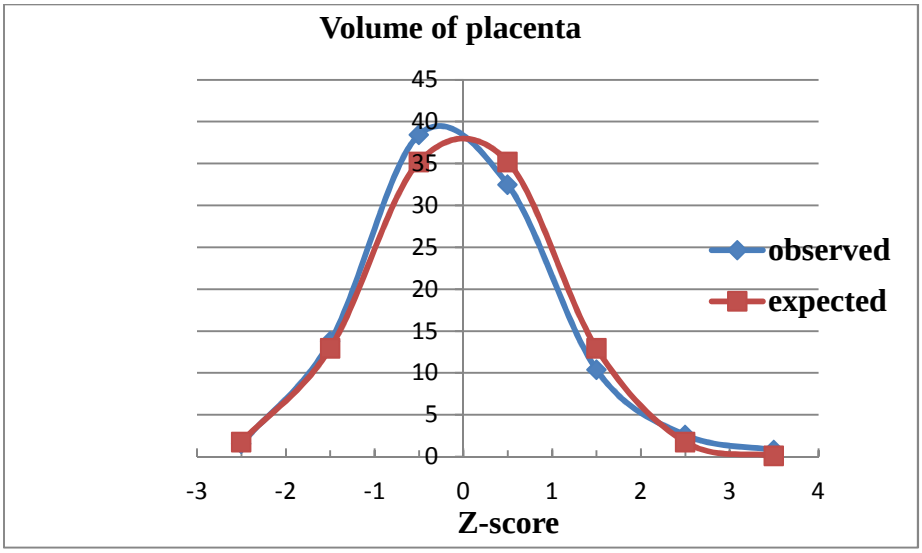

Table 15 and Graph 10 reveal that the percent distribution of the volume of the placenta was positively skewed with mean 415.86 and SD 95.52 ml. In expected data, 50% placenta volume was above the average volume, but in observed data, 46.23% placenta volume was above the average placental volume, the difference was not significant.

Table 16: Percent distribution of the surface area of the placenta

Z-score group of surface area of placenta	Midpoint of z-score group of surface area of placenta	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-3-(-2)	-2.5	6	1.56	1.75
-2(-1)	-1.5	49	12.73	12.95
-1-0	-0.5	156	40.52	35.2
0-1	0.5	105	27.27	35.2
1-2	1.5	57	14.81	12.95
2-3	2.5	10	2.6	1.75
3+	3.5	2	0.52	0.09
Total		**385**	**100**	**100**
Mean= 236.46, SD= 51.38				

Graph 11: Percent distribution of the surface area of the placenta

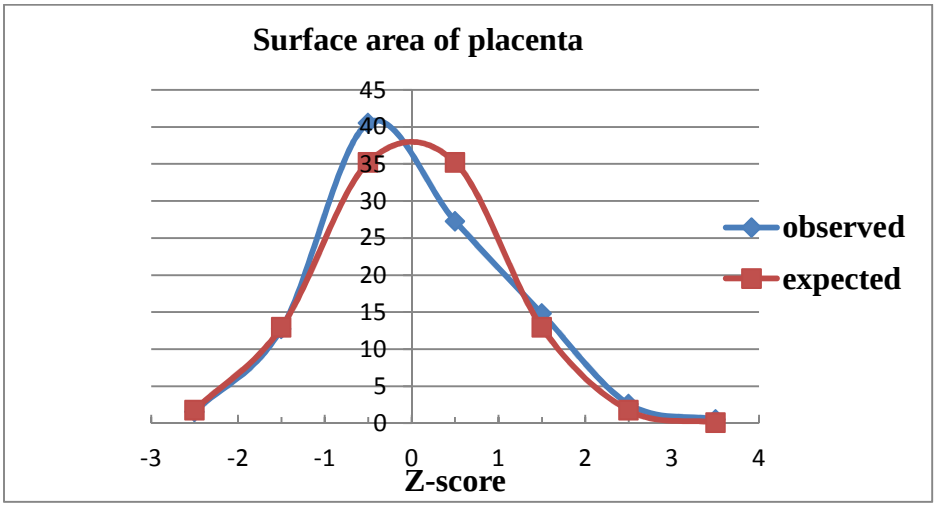

Table 16 and Graph 11 reveal that percent distribution of the surface area of the placenta was positively skewed with mean 236.46 and SD 51.38 cm^2. In expected data, 50% of the surface area of the placenta was above the average, but in observed data, 45.19% were above the average surface area of the placenta, the difference was not significant.

Table 17: Percent distribution of number of cotyledons of the placenta

Z- score group of No. of cotyledons	Midpoint of z- score group of No. of cotyledons	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-3-(-2)	-2.5	4	1.04	1.75
-2-(-1)	-1.5	31	8.05	12.95
-1-0	-0.5	166	43.12	35.2
0-1	0.5	107	27.79	35.2
1-2	1.5	66	17.14	12.95
2-3	2.5	9	2.34	1.75
3+	3.5	2	0.52	0.09
Total		**385**	**100**	**100**
Mean= 19.77, SD= 4.15				

Graph 12: Percent distribution of number of cotyledons of the placenta

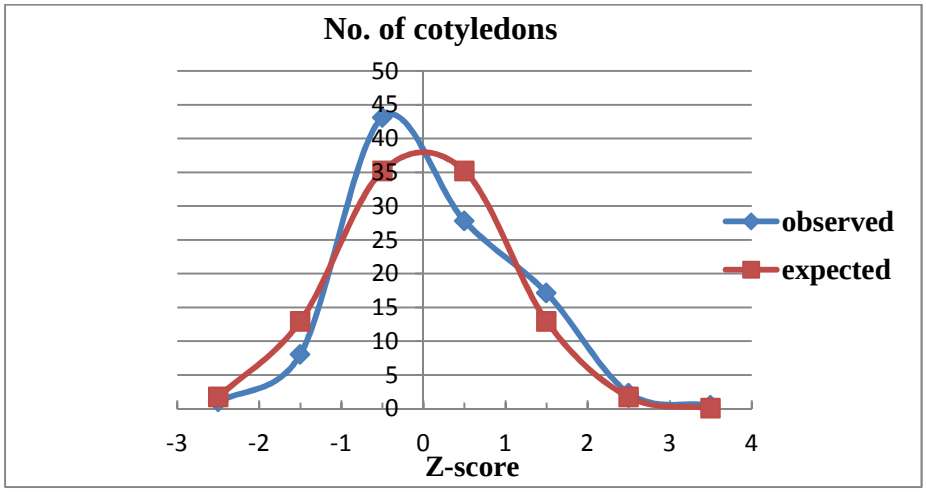

Table 17 and Graph 12 reveal that the percent distribution of cotyledons of the placenta was positively skewed with mean 19.77 and SD 4.15. In expected data, 50% of cotyledons of placenta were above the average No. of cotyledons, but in observed data, 47.79% were above the average No. of cotyledons of the placenta, the difference was significant at $p<0.01$.

Table 18: Percent distribution of No. of blood vessels/villi of the placenta

Z-score group of No. of blood vessels/villi	Midpoint of z-score group of No. of blood vessels/villi	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-3-(-2)	-2.5	3	0.78	1.75
-2-(-1)	-1.5	51	13.25	12.95
-1-0	-0.5	160	41.56	35.2
0-1	0.5	115	29.87	35.2
1-2	1.5	45	11.69	12.95
2-3	2.5	4	1.04	1.75
3+	3.5	7	1.82	0.09
Total		**385**	**100**	100
Mean= 6.73, SD= 1.34				

Graph 13: Percent distribution of No. of blood vessels/villi of the placenta

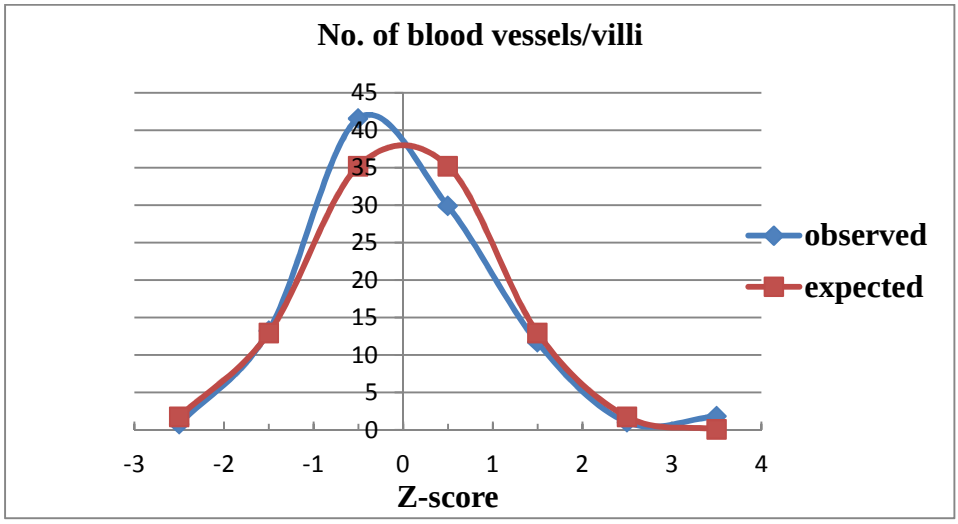

Table 18 and Graph 13 reveal that the percent distribution of No. of blood vessels/villi of the placenta was positively skewed with mean 6.73 and SD 1.34. In expected data, 50% number of blood vessels/villi of the placenta was above the average No. of blood vessels/villi, but in observed data, 44.42% were above the average No. of blood vessels/villi of the placenta, the difference was not significant.

Table 19: Cord attachment of the placenta

Cord attachment	Subjects (n)	%
Central	35	9.091
Eccentric	282	73.25
Marginal	56	14.55
Velamentous	12	3.117
Total	**385**	**100**

Table 19 shows the distribution of cord attachment of the placenta.73.24% of umbilical cord showed eccentric attachment, 14.54% showed marginal attachment, 9.09% showed central and 3.12% showed velamentous attachment.

Table 20: Membrane attachment of the placenta

Type of attachment	Subjects	%
Membranous	305	79.22
Circummarginate	56	14.55
Circumvallate	24	6.23
Grand Total	**385**	**100**

Table 20 shows the distribution of membrane attachment of the placenta. 79.22% of placentae showed membranous attachment, 14.55% showed circummarginate attachment and 6.23% showed circumvallate attachment.

Table 21: Anomalies of the placenta

Anomalies	Subjects (n)	%
No anomalies	211	54.81
Infarction	82	21.30
Calcium salts	43	11.17
Infarction and calcium salts	49	12.73
Total	**385**	**100**

Table 21 shows the distribution of anomalies of the placenta. 54.81% of placentae were normal, 21.30% were infarcted, 11.17% showed calcium salt deposition and 12.73% showed both infarction and calcium salt deposition.

4. Percent distribution of cellular expression of VEGF:

Table 22: Percent distribution of STB cells VEGF

Z-score group of STB cells VEGF	Midpoint of z-score group of STB cells VEGF	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
<-3	-3.5	1	0.26	0.09
-3-(-2)	-2.5	3	0.78	1.75
-2-(-1)	-1.5	52	13.51	12.95
-1-0	-0.5	147	38.18	35.2
0-1	0.5	123	31.95	35.2
1-2	1.5	47	12.21	12.95
2-3	2.5	12	3.12	1.75
Total		**385**	**100**	**100**
Mean= 114.75, SD= 9.44				

Graph 14: Percent distribution of STB cells VEGF

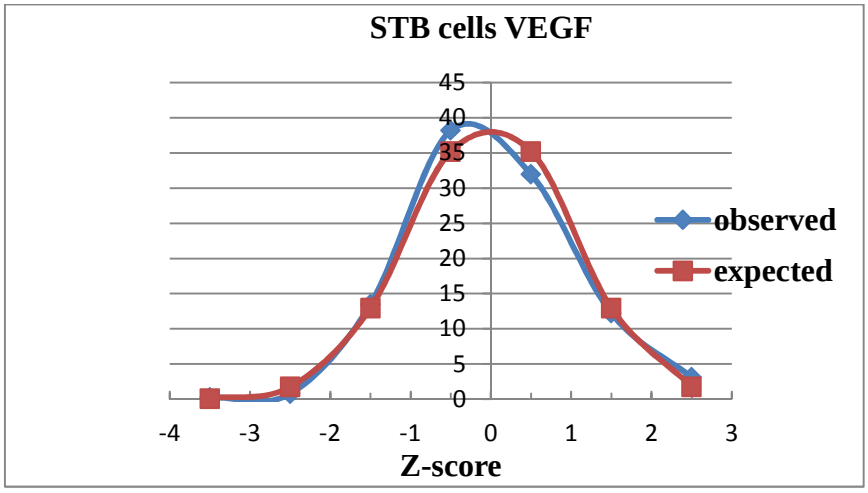

Table 22 and Graph 14 reveal that the percent distribution of STB cells VEGF of the placenta was positively skewed with mean 114.75 and SD 9.44. In expected data, 50% of STB cells VEGF of the placenta was above the average of STB cells VEGF, but in observed data, 47.27% were above the average of STB cells VEGF of the placenta. The difference between them was not statistically significant.

Table 23: Percent distribution of BV VEGF

Z-score group of BV VEGF	Midpoint of z-score group of BV VEGF	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-3-(-2)	-2.5	11	2.86	1.75
-2-(-1)	-1.5	53	13.77	12.95
-1-0	-0.5	111	28.83	35.2
0-1	0.5	159	41.3	35.2
1-2	1.5	43	11.17	12.95
2-3	2.5	8	2.08	1.75
Total		**385**	**100**	**100**
Mean= 117.64, SD= 11.43				

Graph 15: Percent distribution of BV VEGF

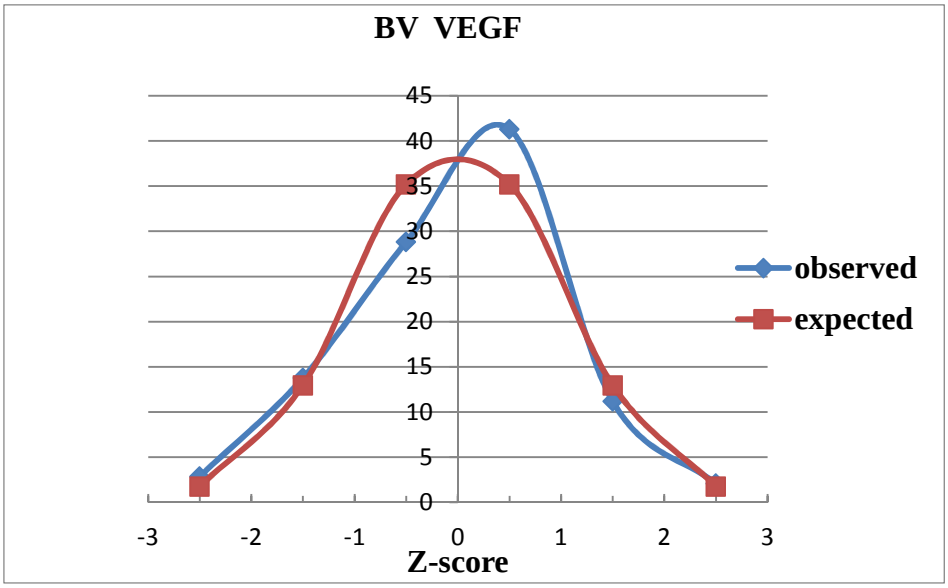

Table 23 and Graph 15 reveal that the percent distribution of BV VEGF of the placenta was negatively skewed with mean 117.64 and SD 11.43. In expected data, 50% of BV VEGF of the placenta was below the average of BV VEGF, but in observed data, 45.45% were below the average of BV VEGF of the placenta. The difference between them was not statistically significant.

Table 24: Percent distribution HC VEGF

Z-score group of HC VEGF	Midpoint of z-score group of HC VEGF	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-3-(-2)	-2.5	12	3.12	1.75
-2-(1)	-1.5	52	13.51	12.95
-1-0	-0.5	131	34.03	35.2
0-1	0.5	130	33.77	35.2
1-2	1.5	50	12.99	12.95
2-3	2.5	8	2.08	1.75
3+	3.5	2	0.52	0.09
Total		**385**	**100**	**100**
Mean= 121.02, SD= 9.34				

Graph 16: Percent distribution of Hofbauer cells VEGF

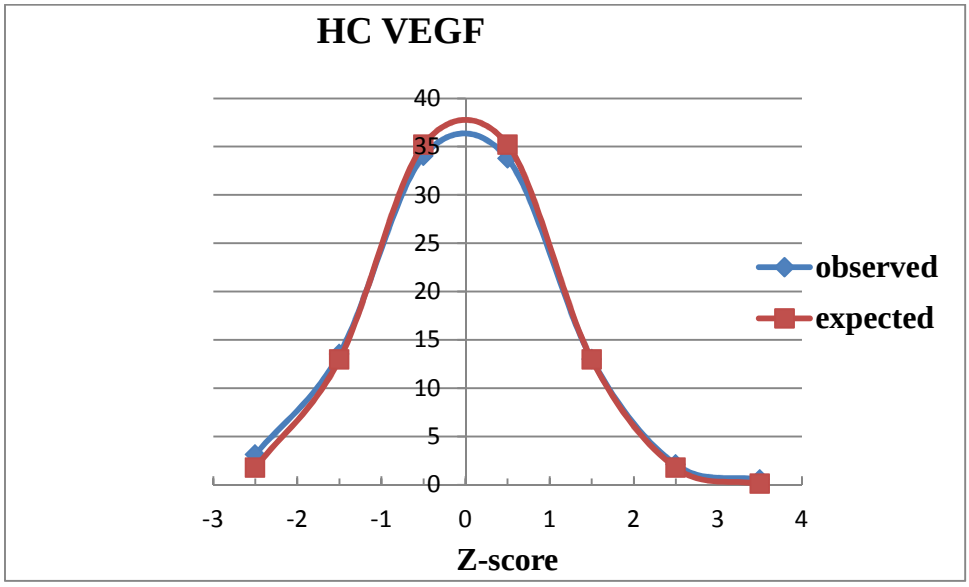

Table 24 and Graph 16 reveal that the percent distribution of HC VEGF of the placenta was normally distributed with mean 121.02 and SD 9.34. There were no differences between the expected and observed distributions.

Table 25: Percent distribution total VEGF

Z-score group of total VEGF	Midpoint of z-score group of total VEGF	Subjects (n)	Frequency distribution	
			Observed (%)	Normal distribution (%)
-3-(-2)	-2.5	4	1.04	1.75
-2-(-1)	-1.5	67	17.4	12.95
-1-0	-0.5	115	29.87	35.2
0-1	0.5	145	37.66	35.2
1-2	1.5	44	11.43	12.95
2-3	2.5	9	2.34	1.75
3+	3.5	1	0.26	0.09
Total		**385**	**100**	**100**
Mean= 117.81, SD= 8.03				

Graph 17: Percent distribution of total VEGF

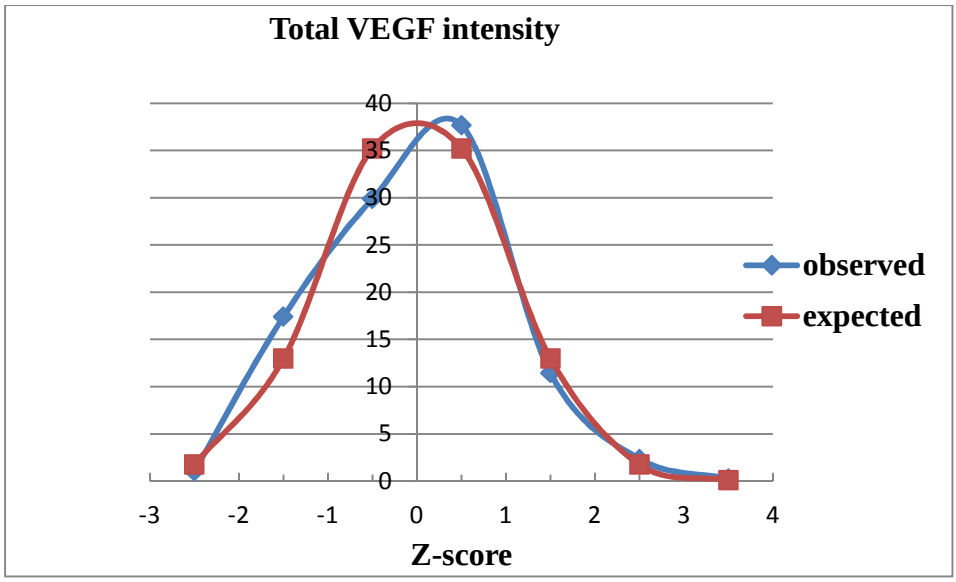

Table 25 and Graph 17 reveal that the percent distribution of total VEGF of the placenta was negatively skewed with mean 117.81 and SD 8.03. In expected data, 50% of the total level of VEGF of the placenta was below the average level of VEGF, but in observed data, 48.31% were below the average level of VEGF of the placenta. The difference between them was not statistically significant.

Expression of VEGF by maternal, fetal and placental growth parameters:

I. Expression of VEGF by maternal parameters:

Expression of VEGF by age of the mother:

Table 26: Expression of VEGF by the age of the mother

Age group of the mother (in years)		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
<20	N	5	5	5	5	NS
	Mean	113.40	114.67	117.97	115.34	
	SD	6.42	7.75	6.23	5.86	
20-24	N	103	103	103	103	HC(p<0.001)
	Mean	113.88	115.45	119.53	116.28	
	SD	9.03	11.83	9.32	8.21	
25-29	N	50	50	50	50	HC(p<0.001)
	Mean	111.47	112.64	120.95	115.02	
	SD	8.78	11.09	6.50	7.17	
30-34	N	10	10	10	10	NS
	Mean	114.47	118.79	117.75	117.00	
	SD	9.97	9.85	11.43	7.67	
35+	N	5	5	5	5	NS
	Mean	115.60	110.57	118.57	114.91	
	SD	14.77	14.89	10.28	12.28	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

(NS-Not significant)

Table 26 shows the expression of VEGF by the age of the mother. The cellular expression of VEGF did not change with the maternal age (19 - 35+). The cellular expression between the cells was more in HC cells than STB and BV, which was significant at p<0.001 in 20 - 24, 25 - 29 and total age group mothers.

Expression of VEGF by parity:

Table 27: Expression of VEGF by parity

Parity		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
Primipara	N	83	83	83	83	HC(p<0.001)
	Mean	112.04	114.51	119.44	115.33	
	SD	8.16	11.70	8.98	7.91	
Multipara	N	90	90	90	90	HC(p<0.001)
	Mean	114.37	114.80	120.06	116.41	
	SD	9.79	11.30	8.28	7.89	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

Table 27 shows the expression of VEGF by parity. The expression of VEGF did not change with the parity. The expression was increased slightly in multipara than primipara, but it was not significant (p>0.05). The cellular expression between the cells was more in HC cells than STB and BV, which was significant at p<0.001 in both the groups.

Expression VEGF by mode of delivery:

Table 28: Expression VEGF by mode of delivery

Type of delivery		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
Normal vaginal	N	102	102	102	102	HC(p<0.001)
	Mean	114.10	116.09	120.42	116.87	
	SD	8.96	11.14	8.20	7.39	
C/S	N	71	71	71	71	HC(p<0.001)
	Mean	112.04	112.62	118.82	114.49	
	SD	9.24	11.83	9.20	8.43	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

Table 28 shows the VEGF expression by mode of delivery. The expression of VEGF did not change with the mode of delivery. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at p<0.001 in both the groups.

Expression of VEGF by the hemoglobin level of the mother:

Table 29: Expression of VEGF by the hemoglobin level of the mother

Hemoglobin group of mother (g/dL)		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
7-10.9	N	77	77	77	77	HC(p<0.001)
	Mean	115.22*	115.70	119.90	116.94	
	SD	9.62	11.30	9.06	8.16	
11+	N	96	96	96	96	HC(p<0.001)
	Mean	111.67	113.83	119.65	115.05	
	SD	8.40	11.68	8.32	7.62	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

Table 29 shows the expression of VEGF by maternal hemoglobin level. VEGF was increased in STB, BV cells and total in 7 - 10.9 g/dL hemoglobin group mothers than the normal hemoglobin group (11+ g/dL). But the rise in STB was significant at $p<0.05$. The VEGF in HC cells did not change. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at $p<0.001$ in both the hemoglobin group.

II. Expression of VEGF by fetal parameters:

Expression of VEGF by gestational age:

Table 30: Expression of VEGF by gestational age

Gestational age group (in weeks)		Intensity of STB	Intensity of BV	Intensity of HC	Total VEGF intensity	p-value
<37	N	25	25	25	25	BV(p<0.05)
	Mean	109.56	116.02	116.65	114.07	HC(p<0.001)
	SD	8.23	10.07	6.54	6.21	
37-38	N	59	59	59	59	
	Mean	113.37	113.93	121.78	116.36	HC(p<0.001)
	SD	11.46	12.42	8.74	9.11	
39-40	N	77	77	77	77	
	Mean	113.41	115.07	117.32	115.26	HC(p<0.001)
	SD	7.41	12.09	7.59	7.65	
41+	N	12	12	12	12	STB(P<0.001)
	Mean	119.33***	112.86***	132 ***	121.40**	HC(p<0.001)
	SD	2.60	4.38	4.73	2.12	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

(***p<0.001, **p<0.01, *p<0.05)

Table 30 shows the expression of VEGF by gestational age. The expression of VEGF did not change with gestation from 32 - 40 weeks of gestation. The VEGF level was increased in 41+ weeks of gestation significantly than 32 - 40 weeks, differences between them were significant at p<0.001. The VEGF was increased in HC cells than STB, BV cells, which was very highly significant at p<0.001 and in BV cells of <37 (p<0.05) and in 41+ STB cells (p<0.001).

Expression of VEGF by sex of the fetus:

Table 31: Expression of VEGF by sex of the fetus

Sex of fetus		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
Male	N	84	84	84	84	HC($p<0.001$)
	Mean	113.30	116.49*	120.23	116.67	
	SD	10.02	12.34	8.35	7.97	
Female	N	89	89	89	89	HC($p<0.001$)
	Mean	113.20	112.94	119.33	115.15	
	SD	8.22	10.46	8.91	7.80	
Total	**N**	**173**	**173**	**173**	**173**	HC($p<0.001$)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

(*$p<0.05$)

Table 31 shows the VEGF expression by sex of the fetus. The VEGF expression was increased in BV in male than a female fetus, which was significant at $p<0.05$. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at $p<0.001$ in both the groups.

Expression of VEGF by birth weight:

Table 32: Expression of VEGF by birth weight

Birth weight group (in gm)		Intensity of STB	Intensity of BV	Intensity of HC	Total VEGF intensity	p-value
2000-2499	N	36	36	36	36	HC($p<0.01$)
	Mean	113.84	116.75	119.67	116.75	
	SD	10.41	7.93	8.22	6.79	
2500-2999	N	90	90	90	90	HC($p<0.001$)
	Mean	112.03	114.76	119.70	115.49	
	SD	8.25	12.05	8.47	7.49	
3000+	N	47	47	47	47	HC($p<0.001$)
	Mean	115.15	112.89	119.96	116.00	
	SD	9.19	12.68	8.67	9.21	
Total	**N**	**173**	**173**	**173**	**173**	HC($p<0.001$)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

Table 32 shows the expression of VEGF by birth weight. The VEGF expression did not change with the birth weight. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at $p<0.001$ in 2500 - 2999 and 3000+ group, and 2000 - 2499 at $p<0.01$.

Expression of VEGF by the length of the fetus:

Table 33: Expression of VEGF by the length of the fetus

Fetal length group (in cm)		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
<45	N	17	17	17	17	HC($p<0.05$)
	Mean	113.90	116.14	121.34	117.13	
	SD	11.07	10.80	8.49	7.92	
45-56	N	156	156	156	156	HC($p<0.001$)
	Mean	113.18	114.50	119.59	115.76	
	SD	8.70	11.59	8.62	7.85	
Total	**N**	**173**	**173**	**173**	**173**	HC($p<0.001$)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

Table 33 shows the expression of VEGF by the length of the fetus. The VEGF expression did not change with the length. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at $p<0.001$ in the 45 - 56 length group and in <45 at $p<0.05$.

Expression of VEGF by head circumference:

Table 34: Expression of VEGF by head circumference

Head circumference group (in cm)		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
<32	N	35	35	35	35	HC(p<0.001)
	Mean	112.85	116.08	119.09	116.01	
	SD	9.61	9.89	6.73	6.68	
33-35	N	117	117	117	117	HC(p<0.001)
	Mean	112.80	114.33	119.62	115.58	
	SD	8.53	12.07	8.97	8.10	
36+	N	21	21	21	21	HC(p<0.05)
	Mean	116.44	114.14	121.69	117.42	
	SD	11.08	11.20	9.63	8.76	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

Table 34 shows the expression of VEGF by head circumference. The VEGF did not change with the head circumference. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at p<0.001 in <32, 33 - 35 and in total groups and 36+ at p<0.05.

III. Expression of VEGF by placental parameters:

Expression of VEGF by placental weight:

Table 35: Expression of VEGF by placental weight

Placenta weight group (in cm)		Intensity of STB	Intensity of BV	Intensity of HC	Total VEGF intensity	p-value
<400	N	50	50	50	50	HC(p<0.01)
	Mean	113.25	115.77	118.42	115.81	
	SD	9.49	12.67	9.86	8.42	
400-600	N	114	114	114	114	HC(p<0.001)
	Mean	113.20	114.61	120.33	116.04	
	SD	8.98	10.70	7.92	7.51	
600+	N	9	9	9	9	HC(p<0.05)
	Mean	113.92	109.27	120.10	114.43	
	SD	9.46	13.43	9.57	9.78	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

Table 35 shows the expression of VEGF by placental weight. The VEGF expression did not change with the placental weight. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at p<0.001 in 400 - 600 and in total and in <400 at p<0.01 and in 600+ at p<0.05.

Expression of VEGF by placental volume:

Table 36: Expression of VEGF by placental volume

Placenta volume group (in ml)		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
<400	N	57	57	57	57	HC($p<0.05$)
	Mean	113.90	117.05	118.28	116.41	
	SD	9.80	12.13	8.71	7.93	
400-600	N	105	105	105	105	HC($p<0.001$)
	Mean	112.58	113.57	120.23	115.46	
	SD	8.64	10.82	8.26	7.65	
600+	N	10	10	10	10	NS
	Mean	115.34	111.43	121.45	116.07	
	SD	9.73	12.91	9.97	9.55	
Total	**N**	**172**	**172**	**172**	**172**	HC($p<0.001$)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.89**	

Table 36 shows the expression of VEGF by placental volume. The VEGF expression did not change with the placental weight. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at $p<0.001$ in 400 - 600 and in total and in <400 at $p<0.05$ and in 600+ though the expression was increased, it was not statistically significant.

Expression of VEGF by placental surface area:

Table 37: Expression of VEGF by the placental surface area

Placental surface area (in $cm^{2)}$		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
<200	N	26	26	26	26	NS
	Mean	112.36	114.84	116.73	114.31	
	SD	9.88	14.59	8.79	8.88	
200-300	N	121	121	121	121	HC(p<0.001)
	Mean	113.37	114.93	120.42	116.24	
	SD	9.20	11.27	8.47	7.86	
300+	N	25	25	25	25	HC(p<0.01)
	Mean	114.12	113.35	120.92	116.13	
	SD	7.56	9.37	8.53	7.07	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	
		26	**26**	**26**	**26**	

Table 37 shows the expression of VEGF by the placental surface area. VEGF expression did not change with the placental surface area. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at p<0.001 in 200 – 300 and in total and in 300+ at p<0.01 and in <200 though the expression was increased, it was not statistically significant.

Expression of VEGF by the number of cotyledons of the placenta:

Table 38: Expression of VEGF by the number of cotyledons of the placenta

Cotyledons group		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
10-25	N	145	145	145	145	HC($p<0.001$)
	Mean	112.87	114.83	119.27	115.66	
	SD	9.27	11.93	8.63	8.08	
25+	N	28	28	28	28	HC($p<0.001$)
	Mean	115.23	113.82	122.29	117.11	
	SD	7.85	8.93	8.17	6.87	
Total	**N**	**173**	**173**	**173**	**173**	HC($p<0.001$)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

Table 38 shows the expression of VEGF by the number of cotyledons of the placenta. The VEGF expression did not change with the number of placental cotyledons. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at $p<0.001$ in all the groups.

Expression of VEGF by umbilical cord attachment:

Table 39: Expression of VEGF by umbilical cord attachment

Umbilical cord attachment		Intensity of STB	Intensity of BV	Intensity of HC	Total VEGF intensity	p-value
Central	N	15	15	15	15	NS
	Mean	116.15	119.40	123.14	119.56	
	SD	11.34	10.22	9.23	7.78	
Eccentric	N	127	127	127	127	HC(p<0.001)
	Mean	113.32	114.70	119.18	115.73	
	SD	8.18	11.78	8.69	7.73	
Marginal	N	26	26	26	26	HC(p<0.001)
	Mean	110.79	112.47*	121.14	114.80	
	SD	10.84	10.14	7.91	7.99	
Velamentous	N	5	5	5	5	NS
	Mean	115.75	110.92	117.36	114.68	
	SD	14.07	13.95	8.01	11.39	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

(*p<0.05, NS=not significant)

Table 39 shows the expression of VEGF by attachment of the umbilical cord. The expression of VEGF from normal attachment (central to eccentric) to abnormal (marginal and velamentous) reduced consistently. But the difference between the expression of VEGF from central to marginal attachment was statistically significant in BV cells ($p<0.05$). In the velamentous cord, the VEGF level was less than other types, but we could not conclude anything due to less sample size in that group. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at $p<0.001$ in eccentric and marginal cord attachment and total, and in central and velamentous, though the expression was increased, it was not statistically significant.

Expression of VEGF by membrane attachment of the placenta:

Table 40: Expression of VEGF by membrane attachment of the placenta

Membrane attachment		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
Membranous	N	139	139	139	139	HC(p<0.001)
	Mean	113.39	114.96	119.88	116.07	
	SD	9.40	11.70	8.94	8.05	
Circummarginate	N	23	23	23	23	NS
	Mean	115.01	115.31	119.39	116.56	
	SD	8.17	10.36	8.24	7.68	
Circumvallate	N	11	11	11	11	HC(p<0.001)
	Mean	107.82***	109.62	119.13	112.19*	
	SD	4.54	11.25	5.30	5.68	
Total	**N**	**173**	**173**	**173**	**173**	HC(p<0.001)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

(***p<0.001, *p<0.05, NS=not significant)

Table 40 shows the expression of VEGF by the membrane attachment. The expression of VEGF was reduced consistently from normal membrane attachment to circumvallate. The differences between membranous and circumvallate were significant in STB (p<0.001) and total (p<0.05) expression. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at p<0.001 in membranous, circumvallate and in total and in circummarginate though the expression was increased in HC cells, it was not statistically significant.

Expression of VEGF by the number of blood vessels/villi:

Table 41: Expression of VEGF by the number of blood vessels/villi

Blood vessels group		**Intensity of STB**	**Intensity of BV**	**Intensity of HC**	**Total VEGF intensity**	**p-value**
4-9	N	169	169	169	169	HC($p<0.001$)
	Mean	113.30	114.68	119.69	115.89	
	SD	9.17	11.60	8.61	7.96	
10+	N	4	4	4	4	HC($p<0.05$)
	Mean	110.99	114.16	123.05	116.07	
	SD	5.95	8.53	8.96	5.01	
Total	**N**	**173**	**173**	**173**	**173**	HC($p<0.001$)
	Mean	**113.25**	**114.66**	**119.76**	**115.89**	
	SD	**9.11**	**11.52**	**8.63**	**7.90**	

Table 41 shows the expression of VEGF by the number of blood vessels/terminal villi. The VEGF did not change with the number of blood vessels/terminal villi. The cellular expression between the cells was more in HC cells than STB and BV, which was significant at $p<0.001$ in 4-9 and in total and in 10+ at $p<0.05$.

IV. Expression of VEGF in complicated pregnancies:

Table 42: Expression of VEGF in complicated pregnancies

Case	Subjects	Intensity of STB		Intensity of BV		Intensity of HC		Total VEGF intensity		p-value
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Normal	70	111.97	8.29	112.56	12.38	119.98	8.54	114.84	8.13	HC(p<0.001)
PE	33	117.89***	9.58	123.97***	10.81	121.99	9.84	121.28***	8.09	BV(p<0.05)
Eclampsia	7	118.26	9.57	115.01	12.38	119.56	10.35	117.61	9.37	NS
GHP	23	112.32	8.76	117.68*	7.77	121.31	8.50	117.11	6.90	BV(p<0.05) HC(P<0.001)
IUGR	30	121.35***	10.36	124.69***	10.32	123.02	7.97	123.02***	7.42	NS
GDM	13	115.45	12.22	125.24***	13.59	123.30	14.06	121.33	11.66	NS
Anemia	41	115.31	9.77	118.84**	9.99	121.74	7.58	118.63**	6.95	HC(P<0.001)
Hypothyroid	42	115.62*	8.58	119.44**	11.93	123.59*	8.28	119.55***	7.67	HC(P<0.001)
APH	10	110.97	10.20	121.85*	10.78	120	9.97	117.61	8.79	BV(p<0.05) HC(p<0.05)
PROM	52	118.68***	7.96	117*	11.41	122.74	10.89	119.48***	8.06	HC(P<0.01)
Oligohydramnios	29	118.48***	8.43	122.24***	8.68	122.60	6.21	121.11***	5.10	HC(P<0.05)
Congenital anomalies	18	113.30	10.65	125.38***	10.62	123.53	10.11	120.74***	7.55	BV(p<0.001) HC(p<0.01)
Low birth weight	92	114.73*	8.93	118.45***	9.09	120.89	8.50	118.02**	6.63	BV(p<0.01) HC(P<0.001)
Polyhydramnios	9	113.78	5.53	122.22**	8.71	121.93	8.53	119.31*	5.64	BV(p<0.05) HC(p<0.05)

(***p<0.001,**p<0.01,*p<0.05,NS=not significant)

Table 42 shows the VEGF expression in normal and complicated pregnancies. The cellular expression was more in HC cells of normal than STB and BV cells, which was significant at $p<0.001$.

In PE and IUGR placenta, the VEGF was increased significantly in all the cells and in total than the normal, which was very highly significant at $p<0.0001$ in STB, BV and total. Though the expression was increased in the HC cell, it was not significant. In PE, comparing the cellular expression between the cells, we found the expression was more in BV cells which was significant at $p<0.05$. In IUGR, the expression level was not significant between the cells.

In eclampsia, though the expression level was increased than the normal, it was not statistically significant and the difference between the cell group was also not significant.

In GHP, the VEGF level was increased in all the cells and was statistically significant only in BV cells ($p<0.05$) of GHP than the normal. In GHP, when the comparison was made between three cells, the cellular expression was found more in HC ($p<0.001$) and BV cells ($p<0.05$).

In GDM, the expression level of VEGF was increased in all the cells compared to normal, was significantly high in BV cells at $p<0.0001$ than the normal. The difference between the cell group was not significant.

In anemia, the expression of VEGF was increased in all the cells, but a significant difference was observed in BV and total, and was highly significant at $p<0.01$. When cells were compared for expression between the cells, the cellular expression was found more in HC cells ($p<0.001$).

In hypothyroid, the VEGF was significantly increased in STB , HC cells ($p<0.05$), BV cells ($p<0.05$) and in total ($p<0.001$) than normal. When cells were

compared for expression between the cells, the cellular expression was found to be more in HC cells ($p<0.001$).

In APH, the expression was significantly increased in BV, HC, and in total, but significantly increased in BV cells ($p<0.05$). The cellular expression between the cells was more in HC and BV cells ($p<0.05$).

In PROM, the expression was very highly significant in STB and total ($p<0.001$) and was significant in BV cells ($p<0.05$), though the expression was more in HC cells, it was not significant, but when comparing the expression between cells, HC cells exhibited a significant rise ($p<0.01$).

In oligohydramnios, the VEGF increased in STB, BV and in total, which was significant at $p<0.001$, though the expression was more in HC cells, it was not significant, but when comparing the expression between cells, HC cells displayed a significant rise ($p<0.05$).

In a congenital anomaly, the expression was very highly significant in BV and total ($p<0.001$), though the expression was more in HC and STB cells, it was not significant. When we compared expression between the cells, BV cells showed more expression at $p<0.001$ and HC cells at $p<0.01$.

In low birth, the VEGF increased in BV ($p<0.001$), total ($p<0.01$) and in STB ($p<0.05$), though the expression was more in HC cells, it was not significant. The expression between the cells was increased in HC ($p<0.001$) and BV cells ($p<0.01$).

In polyhydramnios, the VEGF was significantly high in BV cells ($p<0.01$) and in total ($p<0.05$), though the level was more in HC and STB cells, which was not significant. The expression between the cell group BV and HC cells showed more intensity ($p<0.05$).

5. DISCUSSION

The present study was conducted in the department of Anatomy. The samples were collected from the Obstetrics and Gynaecology Unit, Dr Prabakar Kore Charitable Hospital, Belagavi. The study aimed to check the expression of VEGF in the placenta and its association with growth parameters to study its role in growth. To study the role of VEGF in the growth of the placenta and fetus, we compared the expression of VEGF with morphometrical variables of the placenta and fetus. The VEGF in the placenta helps in the sprouting of first blood vessels. The development of vessels is important in the transport of nutrition between maternal circulation and placenta, which determines the growth of the placenta and thereby the fetus. We, therefore, presumed that the VEGF stimulates the growth of the placenta by increasing branching (vessel formation) and non-branching angiogenesis thereby increasing surface area and vascular permeability for nutrition transport, which is reflected through placental growth which in turn increases the fetal growth.

Expression of VEGF by maternal parameters:

Expression of VEGF by maternal age:

The cellular expression of VEGF did not change with the maternal age (19 - 35+). A serum analysis study showed that the VEGF level at 8 - 20 weeks of gestation did not correlate with maternal age.[48] By these results, we can say that VEGF expression was not affected by maternal age.

Expression of VEGF by parity and gravidity:

In this study, VEGF expression did not change with parity and gravidity. A study showed that serum VEGF level did not change with the parity of the mother at 8 - 20 weeks of pregnancy.[48] Therefore, the VEGF expression was not affected by the parity and gravidity of the mother.

Expression of the VEGF by mode of delivery:

The expression of VEGF did not change with the mode of delivery. Malamitsi Pucher A et al have reported similar findings with serum level VEGF at term. By our results, we can say that VEGF expression was not affected by the mode of delivery.[140]

Expression of VEGF by the hemoglobin level of the mother:

The expression of VEGF was increased in STB, BV cells and total in 7 - 10.9 g/dL hemoglobin group mothers than the normal hemoglobin group (11+ g/dL). But the VEGF expression in STB was increased significantly in 7 - 10.9 g/dL hemoglobin group mothers ($p<0.05$). The low hemoglobin level decreases the oxygen content of the blood. VEGF is a hypoxia-inducible factor. The increased expression in this study was due to hypoxia-induced VEGF secretion.[141]

Expression of VEGF by fetal parameters:

Expression of VEGF by gestational age:

In this study, the VEGF expression did not change from 32 - 40 weeks of pregnancy. In >37 weeks of gestation, the VEGF level was 109.56 ± 8.23 in STB, in BV it was 116.02 ± 10.07, in HC it was 116.65 ± 6.54 and the total expression was 114.07 ± 6.21. In 37 - 38 weeks of gestation, the VEGF level in STB was 113.37 ±

11.46, in BV it was 113.93 ± 12.42, in HC it was 121.78 ± 9.11 and the total was 116.36 ± 9.11. In 39 - 40 weeks of gestation, the VEGF level in STB was 113.41 ± 7.41, in BV it was 115.07 ± 12.09, in HC it was 115.26 ± 7.65 and the total was 115.26 ±7.65. In 41+ weeks of gestation, the expression was increased significantly compared to 32 - 40 weeks of pregnancy. The VEGF level in STB was 119.33 ± 2.60, in BV it was 112.86 ± 4.38, in HC it was 132 ± 4.73 and the total was 121.40 ± 2.12. The VEGF expression was increased significantly in 41+ weeks compared to 32 - 40 weeks, the rise in the expression was statistically significant ($p<0.001$). As the gestational age advances, blood vessel formation increases, the oxygen level becomes stable through vasodilation of blood vessels by NO.[142] At the end of pregnancy, blood vessel formation was stabilized and sufficient oxygen supply may be the reason why we did not see changes in the level of VEGF in 32 - 40 weeks of gestation. VEGF was increased in 41+ weeks, may be due to placental insufficiency. Because after 40 weeks of gestation, calcium and fibrin start depositing on villi which limits the blood supply.[143] A histological study also showed underperfused villi in the placenta of prolonged pregnancy.[144] This reduces amniotic fluid volume, which compresses the umbilical cord.[145-147] So, there was hypoxia created by placental insufficiency. The increased expression of VEGF in 40+ weeks of gestation in this study was, may be due to hypoxia created by placental insufficiency.

A study showed that the VEGF was regulated differentially depending upon the physiological needs of the mother and fetus throughout gestation.[148]

Expression of VEGF by sex of fetus:

VEGF was increased in the male fetus. The expression in the BV cell was 116.49 ± 12.34 in males and 112.94 ± 10.46 in females, the rise in VEGF was

statistically significant ($p<0.05$). One of the studies showed increased angiogenic factors (PlGF, VEGF) in women carrying a male fetus. Male baby carrying women show more pro-angiogenic response.[149] Our study also agrees with these reports that VEGF expression was affected by the sex of fetus.

Expression of VEGF by fetal morphometrical parameters:

VEGF expression did not change with the birth weight, length, head and abdominal circumference of the fetus from 32 - 41+ weeks of gestation. Sundrani et al in their longitudinal study of angiogenic factors and their association with fetal growth measures in normal pregnancy showed that VEGF expression at term, the average of gestational age was 39.36 ± 1.1 weeks, was not correlated with birth weight, length and head circumference of the fetus. But they found that a correlation between early expression VEGF with birth weight at 16 - 20 weeks of pregnancy and with the length at 26 - 30 weeks of pregnancy.[148]

In a study of angiogenic factors and their association with birth outcomes showed that VEGF expression in normal (gestational age 39 ± 1 weeks) and in PE (gestational age 38 ± 1 weeks) did not show association with birth weight, fetal length, head and chest circumference.[150]

In another study of basic fibroblast growth factor and VEGF in early neonatal life found that serum level of VEGF at term [an average of gestational age was 38.8 ± 1.1 weeks (range, 37 - 40 weeks)] did not show association with birth weight.[140,151]

A study also found no association between fetal sizes with VEGF-A expression in a pig.[152]

These studies did not mention the molecule of VEGF studied and all studied plasma and serum level VEGF at term were from 37 - 40 weeks. But in this study, we studied VEGF-A165 molecule and was an immunohistochemical study of placental tissue. Our study was focused on one molecule i.e. VEGF-A165. In serum and plasma, other molecules of VEGF were also present so we cannot say their exact role. From our study, we can say that fetal growth from 32 - 41+ weeks of gestation was not associated with placental expression of VEGF-A165 molecule.

Expression of VEGF by placental morphometrical parameters:

VEGF expression from 32 - 41+ weeks of gestation did not change with the placental weight, volume, surface area and number of cotyledons from 32 - 41+ weeks of gestation. Our study confirms fetal morphometrical findings because fetal growth is a reflection of placental weight,[153] volume, surface area and several cotyledons of the placenta. The relationship of the placenta and newborn birth weight is 1: 6, which implies that fetal development relies on placental function and development.[154]

Expression of VEGF by attachment of umbilical cord:

The expression of VEGF was reduced significantly as the cord becomes noncentral [from central (119.40 ± 10.22) to marginal (112.47 ± 10.14) attachment] consistently which was significant at $p<0.05$. In the velamentous cord (110.92 ± 13.95), the expression reduced from central but could not get statistically significant results due to less sample (n= 5) in this group. A study reported that abnormal umbilical cord attached placentae had sparse blood vessels, this we can correlate with the reduced VEGF.[155] Another study also reported reduced VEGF expression in

anatomical malformation of the umbilical cord and abnormal cord attachment (marginal). This research demonstrated that VEGF was involved in umbilical cord development.[156] From our result we can confirm that reduced VEGF caused abnormal umbilical cord attachment.

Expression of VEGF by membrane attachment of placenta:

The VEGF was reduced prominently in the circummarginate and circumvallate type of membrane attachment of placentae than membranous. With this result and above abnormal umbilical cord attachment result, we can say that reduced VEGF affected the morphology of the placenta.

Expression of VEGF by the number of blood vessels/villi of the placenta:

The VEGF expression did not change with the number of vessels. By this result, we can say that after 32 weeks of gestation, VEGF did not affect the number of vessels. The VEGF helps in maintaining the blood vessels and vascular permeability than angiogenesis.[60]

Expression of VEGF in complicated pregnancies:

The VEGF was increased significantly in PE (121.28 ± 8.09) and IUGR (123.02 ± 7.42) cases compared to other complicated cases and the normal (114.84 ± 8.13), which was very highly significant at $p<0.001$. A study by Vassilis T showed increased VEGF mRNA levels in placental tissue and plasma of PE and IUGR cases compared to control. Maternal free and bound VEGF-A was higher in PE and IUGR compared to control. They also found that sflt was more in PE and IUGR.[88] Another immunohistochemical studies also reported increased expression of VEGF in PE placentae.[83,92]

In GHP, the expression of VEGF was increased in all the cells and was statistically significant only in BV cells (117.68 ± 7.77, $p<0.05$) of GHP than the normal. A study showed increased expression of VEGF in STB cells of the placenta and concluded that the increased VEGF can be used as a biomarker of hypertensive disorders of pregnancy.[89] Another serum analyses study also showed increased expression of VEGF in pregnancy-induced hypertension.[157]

In pregnancy-induced hypertensive diseases and IUGR pregnancies, there is an inadequate invasion of trophoblast cells, this leads to failure in spiral artery remodelling resulting in poor perfusion and hypoxia.[158-161] The VEGF is a hypoxia-inducible factor. The increase in VEGF in these cases was, may be due to hypoxia.

In GDM cases, the expression of VEGF increased in BV cells significantly (125.24 ± 13.59, $p<0.001$). A study showed increased VEGF in diabetic rats.[77] But, in another study, the expression was normal in hyperglycemic mothers and increased in overt diabetic mothers.[80] The impaired glucose level may be the reason for the increase of VEGF in GDM pregnancies.[79, 161,162]

The expression of VEGF increased significantly in hypothyroid (119.55 ± 7.67) and anemia (118.63 ± 6.95) cases compared to normal (114.84 ± 8.13). A study showed avascular villi, fibrin deposition, increased syncytial knots, cytotrophoblast cell proliferation and stromal fibrosis in anemia placenta. This study showed that morphological changes were a sign of chronic hypoxia and placental insufficiency.[163] Even hypothyroidism causes morphological and histochemical changes in the placenta.[164,165]

In hypothyroid and anemia, both cause structural changes in the placenta that lead to placental insufficiency. The increased expression was, may be due to a compensatory mechanism.

The VEGF expression was also increased significantly in the PROM (119.48 ± 8.06) cases. The role of VEGF in amnion and fetal membranes is still unknown.

A study also showed increased expression of VEGF in preterm PROM pregnancy in fetal membranes.[166] Similar results were found in cord blood of term and preterm rupture of membranes.[167] This study showed that the elevated VEGF was, may be from the placenta or fetus.[167] In another study, increased expression of VEGF was also observed in the decidua.[166] From previous reports and our study, we can say that the rupture of the membrane was regulated by VEGF-A from the fetal membrane, decidua, and placenta together. Both fetal and maternal interaction regulates the PROM.[166] The mechanism behind the increased VEGF-A in PROM needs to be explored.

The VEGF expression was increased in both oligohydramnios and polyhydramnios cases significantly. A study showed that esophageal ligation in sheep increased expressions of VEGF. This study concluded that increased VEGF stimulates vascularity and permeability which may cause polyhydramnios.[168] Another study also found increased VEGF in oligohydramnios in rat models.[169] These results clearly show that VEGF has a role in maintaining amniotic fluid quantity.[170] The mechanisms behind this need to be explored.

The VEGF level was increased significantly in low birth placentae. A study also showed increased serum VEGF in small birth at term babies.[171] We also noticed a

rise in the VEGF level in the placentae of congenital anomaly cases. Carmeliet P and Ferrara N showed that the absence of the VEGF gene caused abnormal angiogenesis, defect in heart development and defect in the development of vessels in organs and placenta.[33,34] So, we can say that alterations in VEGF level can cause birth defects.

Our study showed an increase in the VEGF level in all the complicated pregnancies. Many studies concentrated on identifying VEGF as a marker of PE and IUGR pregnancies. But there is a lack of reports on the role of VEGF in all other complicated pregnancies. The increase in VEGF was, may be compensatory because hypoxia is common in all pregnancy-related disorders.[172,173] Similar studies may be required to understand the mechanisms behind the increase in VEGF in these complications.

6. CONCLUSION

VEGF is an angiogenic growth factor that helps in blood vessel formation in the placenta and decidua, thereby helping in nutrition transport and growth of the fetus. In this study, we studied the role of VEGF in the growth of the fetus by comparing the placental and fetal parameters with VEGF expression from 32 - 41+ weeks of pregnancy. The VEGF expression was stabilized in the third trimester of pregnancy. The expression was increased in 41+ weeks due to placental insufficiency. The VEGF did not show a relationship with fetal and placental growth measures. Therefore, from our study, we can say that VEGF was not associated with growth, but its stabilized expression was important in maintaining pregnancy. Hence VEGF level at term cannot be useful in predicting the fetal growth parameters. Also, the VEGF expression was not influenced by maternal factors. But it was dependent on the sex of the fetus and hemoglobin level of mothers.

The VEGF level was reduced in the abnormal umbilical cord and membranous attachment of placentae. The reduced VEGF level may cause morphological malformations of the placenta. So VEGF may be involved in the development of umbilical cord and fetal membranes.

The VEGF was increased in all the complicated pregnancies, significantly in PE and IUGR. Many studies have been limited to the role of VEGF in PE and IUGR pregnancies. In our study, we observed that VEGF was increased significantly in many complications not only in PE and IUGR. Therefore, from this study, we can say that VEGF alone cannot be useful in predicting the complications. Combinations with other markers will be beneficial in differentiating multiple complications. The

molecular mechanisms behind this increase in the VEGF level in these complications need to be explored.

Limitations and recommendations:

1. This is an immunohistochemical study of the placenta; there is a lack of literature on immunohistochemical results to compare the VEGF level with growth parameters. Similar studies may be required to confirm our results.
2. The VEGF level was increased in all the complicated pregnancies. Further studies can be conducted to understand the molecular mechanisms behind this as there are no studies so far explaining its role in complicated pregnancies other than PE and IUGR cases.

7. SUMMARY

VEGF is a dominant angiogenic growth factor, prominently expressed in the placenta. VEGF stimulates the proliferation of CT cells for the invasion of decidua and spiral artery remodelling thereby helping in initiating uteroplacental circulation. This is also a vascular permeability factor that increases vascular permeability by stimulating NO secretion. NO is a vasodilator that reduces the peripheral resistance of the maternal vessel and increases the cardiac output thereby increasing the nutrition transport and growth of the fetus. The early expression level of VEGF was correlated with a rise in the number of placental and endometrial vessels with placental and fetal weight. In later stages of pregnancy, it helps in nonbranching angiogenesis. But there is a lack of literature on its role in the growth of the placenta and fetus in later stages of pregnancy. The present research was therefore intended to study the placental VEGF in later pregnancy and its relationship with growth parameters. The present study was carried out in the department of Anatomy J. N. Medical College, Belagavi. Samples were collected from the Obstetrics and Gynaecology Unit, Dr Prabhakar Kore Charitable Hospital, Belagavi. The sample size was 385. Placentae of singleton pregnancy of 32 - 41+ weeks of gestation were collected. The placentae were observed for the attachment of cord and membranes, then a central section was taken and processed for immunohistochemistry and H and E staining. The slides were stained with VEGF-A165 mouse monoclonal antibody. Photos of the stained slides were taken and intensity was quantified using the Image J Software.

Expression of VEGF-A was observed in STB, BV and HC cells. The mean and SD of the intensity of these cells were tabulated. To check the goodness of data, the chi-square test was applied by taking expected and observed frequencies. Using the midpoint of z-score, observed and expected frequencies, normal distribution curve

was prepared to check whether it was normally distributed or not. The z-test was applied to check the differences in the VEGF level with the group of each variable. The expression of VEGF in complicated pregnancies was tabulated and studied separately.

The VEGF level did not change from 32 - 40 weeks of gestation. The expression was increased significantly in 41+ weeks of gestation due to hypoxia created by placental insufficiency in prolonged pregnancy.

The expression of VEGF was compared with maternal age, mode of the delivery gravidity and parity. The expression of VEGF did not change with these parameters of the mother. But the mothers with less hemoglobin levels showed a significant increase in the VEGF. This was, may be due to hypoxia created by less oxygen.

To find the role of VEGF in the growth of the placenta and fetus, we compared placental and fetal morphometrical parameters with the VEGF level of 32 - 41+ weeks of gestation. The expression did not show any relationship with the placental and fetal growth parameters. But the VEGF level was dependent on the sex of the fetus. The male fetus showed increased placental VEGF expression due to more proangiogenic response than the female fetus.

We also observed decreased expression of VEGF with the abnormal umbilical cord and membranous attachment of the placenta.

The expression of VEGF was also studied in complicated pregnancies. The expression level was more in PE and IUGR cases than all other complications. But the expression was increased significantly in all the complicated pregnancies compared to normal cases.

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ANNEXURE – I – PROFORMA

PROFORMA

Title of the project: Immunohistochemical Evaluation of Vascular Endothelial Growth Factor-A (VEGF-A) in Human Placenta and Its Role in Growth of Placenta and Fetus

Sr.no. **Admission date:**

Registration Number:

Sociodemographic Information:

Sr.no	Variables with codes	Responded code	Remarks
1	Name, Address, Mobile number		
2	Age		
5	Religion: 1.Hindu 2. Muslim 3. Christian 4.Jain 5. others		
6	Marital relations: 1.Consanguineous 2. Non consanguineous		

Life style and exposure:

Sr.no	Variables with codes	Responded codes	Remarks
7	Habits - 1. Tabacco chewing 2. Alcohol 3. Drug addiction		
8	Occupation 1. Agriculture labour 2. Cultivator 3.Household duties 4. Employee 5.Employer 6.Nonworker 7.Rentier 8.Student 9. Nonworker		
9	Food Habits: 1 Vegetarian a) Lacto b) Ovo vegetarian c) Vegan 2.Non vegetarian		

Previous pregnancy history:

Sr.no	Variables with codes	Responded code	Remarks
10	Age at marriage (in years)		
11	Age at first pregnancy		
12	Birth interval between earlier pregnancy		
13	Use of contraceptive 1.Yes 2.No		
14	History of spontaneous abortion 1. None 2. One 3. Two 4.Three or more		
15	History of induced abortion 1. None 2. One 3. Two 4.Three or more		
16	History of Pre-eclampsia 1.Yes 2.No		
17	History of Gestational hypertension 1.Yes 2.No		
18	History of Gestational diabetes mellitus 1.Yes 2.No		
19	History of Anemia 1.Yes 2.No		
20	History of any infection 1.Yes 2.No		
21	History of nausea and vomiting 1.Yes 2.No		
22	History of antepartum hemorrhage 1.Yes 2.No		
23	History of normal vaginal delivery 1. None 2. One 3. Two 4.Three or more		
24	History of assisted vaginal delivery 1. None 2. One 3. Two 4.Three or more		
25	History of Cesarean section delivery 1. None 2. One 3. Two 4.Three or more		
26	History of postpartum hemorrhage 1.Yes 2.No		
27	History of still birth delivery 1. None 2. One 3. Two 4.Three or more		
28	History of lowbirth weight delivery 1. None 2. One 3. Two 4.Three or more		
29	History of twins 1.Yes 2.No		
30	History of preterm birth delivery 1. None 2. One 3. Two 4.Three or more		
31	History of neonatal death 1. None 2. One 3. Two 4.Three or more		
32	History of congenital disorder delivery 1.Yes 2.No		

Present pregnancy related information:

Sr.no	Variables with codes	Responded code	Remarks
33	LMP (Date)		
34	EDD (Date)		
35	Type of conception 1. Normal 2. Assisted reproductive techniques		
36	Gravidity		
37	Parity		
38	Birth order		
39	Interval between present and last pregnancy		
40	ANC visit 1. One 2. Two 3. Three 4. Four 5. None		
41	Antepartum hemorrhage 1.Yes 2. No		
42	Premature rupture of membranes 1. Yes 2.No		
43	Pre-eclampsia 1. Yes 2.No		
44	Gestational hypertension 1. Yes 2.No		
45	Gestational diabetes 1. Yes 2.No		
46	Jaundice 1. Yes 2.No		
47	Anemia 1. Yes 2.No		
48	Thyroid disease 1. Yes 2.No		
49	Have you experienced any other disease during pregnancy? 1. Yes (specify) 2. No		
50	Blood group 1. A^+ 2. B^+ 3. O, 4. AB^+, 5.A^- 6.B^- 7. O^- 8.AB^-		
51	HB (gm%)		
52	Folic acid supplements 1. Yes 2.No		
53	Calcium supplements 1. Yes 2.No		

54	Any other medications (Specify)		

Fetal Variables and Delivery information:

Sr.no	Variables with codes	Responded code	Remarks
55	Date of delivery		
56	Weeks of gestation at delivery		
57	Type of delivery 1.Normal vaginal, 2. Assisted vaginal, 3.Cesarian Section		
58	Fetal presentation 1. Vertex 2. Breech 3 Oblique 4. Others		
59	Sex 1.male 2.female		
60	Mode of placental delivery 1.Normal 2.Retained		
61	Placental weight (gm)		
62	Volume of Placenta (ml)		
63	Surface area of Placenta ($cm^{2)}$		
64	Birth weight (gm)		
65	Length (cm)		
66	Head circumference (cm)		
67	Abdominal circumference (cm)		
68	Congenital anomaly		
69	Inborn disease of fetus any (Remarks of doctor)		

Histological and immunohistochemical variables:

Sr.no	Variables	Recordings
70	Cellular expression of VEGF in Placenta	
71	Level of expression of VEGF	
72	Vessels/villi of placenta	

ಫಾರ್ಮ

ಪ್ರಕಲ್ಪದ ಸ್ಥಳ: ಇದ ಮಾಸದಲ್ಲಿಯ ರಕ್ತವಾಹಿನಿಯ ಬೆಳವಣಿಗೆಯ ಆಂಶ-ಎ ಅದರ ಇಮ್ಯುನೊಹಿಸ್ಟೋಕೆಮಿಕಲ್ ಮೌಲ್ಯಮಾಪನ ಮತ್ತು ಮಾಸು ಹಾಗೂ ಭ್ರೂಣದ ಬೆಳವಣಿಗೆಯಲ್ಲಿ ಅದರ ಮಹತ್ವ.

ಸಿ. ನಂ.

ಪ್ರವೇಶ ತಾರೀಖು :
ನೋಂದಣಿ ನಂ. .

ಸಾಮಾಜಿಕ ಲೋಕಸಂಖ್ಯೆಯ ನಿಖರವಾದ ಅಭ್ಯಾಸದ ವಿವರ :

ಅ.ನಂ.	ಸಾಂಕೇತಿಕ ಶಬ್ದಸಹ ಬದಲಾಗುವ ಮಾಹಿತಿ	ಪ್ರತಿಕ್ರಿಯೆ ಸಾಂಕೇತಿಕ ಶಬ್ದಸಹ	ಶರಾ
1	ಹೆಸರು, ವಿಳಾಸ, ಮೋ. ನಂ.		
2	ವಯಸ್ಸು		
5	ಧರ್ಮ : 1) ಹಿಂದು 2) ಮುಸ್ಲಿಮ 3) ಕ್ರಿಶ್ಚನ್ 4) ಜೈನ 5) ಇತರ		
6	ಪ್ರತಿ ವಿಷಯದ ಬಗ್ಗೆ : 1) ರಕ್ತ ಸಂಬಂಧವಿರುವ 2) ರಕ್ತ ಸಂಬಂಧವಿರದ		

ಜೀವನ ಶೈಲಿ ಮತ್ತು ಸಂಸರ್ಗದ ಸಾಧ್ಯತೆ :

ಅ.ನಂ.	ಸಾಂಕೇತಿಕ ಶಬ್ದಸಹ ಬದಲಾಗುವ ಮಾಹಿತಿ	ಪ್ರತಿಕ್ರಿಯೆ ಸಾಂಕೇತಿಕ ಶಬ್ದಸಹ	ಶರಾ
7	ಹವ್ಯಾಸ 1) ತಂಬಾಕು ತಿನ್ನುವದು 2) ಮದ್ಯ 3) ಅಮಲ ಪದಾರ್ಥ ವ್ಯಸನ		
8	ವ್ಯವಸಾಯ : 1) ರೈತ ಕೂಲಿ 2) ಭೇಸಾಯಗಾರ 3) ಮನೆ ಕೆಲಸ 4) ನೌಕರಿ 5) ಮಾಲಕ 6) ಕೆಲಸ ಮಾಡದಿರುವವ 7) ಭಾಡಿಗೆ ಪಡೆಯುವವ (ಮನೆ ಮಾಲಕ) 8) ವಿದ್ಯಾರ್ಥಿ 9) ಕೆಲಸ ಮಾಡದಿರುವವ		
9	ತಿನ್ನುವ ಹವ್ಯಾಸ 1) ಶಾಕಾಹಾರಿ ಅ) ದುಗ್ಧ ಪದಾರ್ಥ ಬ) ಓವಾ ಶಾಕಾಹಾರಿ 2) ವೆಗನ 3) ಮಾಂಸಾಹಾರಿ		

ಹಿಂದಿನ ಗರ್ಭಾವಸ್ಥೆಯ ಇತಿಹಾಸ :

ಅ.ನಂ.	ಸಾಂಕೇತಿಕ ಶಬ್ದಸಹ ಬದಲಾಗುವ ಮಾಹಿತಿ	ಪ್ರತಿಕ್ರಿಯೆ ಸಾಂಕೇತಿಕ ಶಬ್ದಸಹ	ಶರಾ
10	ವಿವಾಹದ ವೇಳೆಯಲ್ಲಿ ವಯಸ್ಸು (ವರ್ಷಗಳಲ್ಲಿ)		
11	ಪ್ರಥಮ ಗರ್ಭಧಾರಣೆಯಲ್ಲಿ ವಯಸ್ಸು		
12	ಹಿಂದಿನ ಗರ್ಭಧಾರಣೆಯಲ್ಲಿಯ ಜನ್ಮದ ಅಂತರ		
13	ಗರ್ಭನಿರೋಧಕಗಳ ಬಳಕೆ 1)ಹೌದು 2) ಇಲ್ಲ		
14	ತನ್ನಿಂದ ತಾನೇ ಗರ್ಭಪಾತವಾದ ಬಗ್ಗೆ : 1) ಒಂದೂ ಇಲ್ಲ 2) ಒಂದು 3) ಎರಡು 4) ಮೂರು ಹಾಗೂ ಹೆಚ್ಚು		
15	ಐಚ್ಛಿಕ ಗರ್ಭಪಾತದ ಘಟನೆ 1) ಒಂದೂ ಇಲ್ಲ 2) ಒಂದೂ 3) ಎರಡು 4) ಮೂರು ಹಾಗೂ ಹೆಚ್ಚು		
16	ಪ್ರಿ-ಎಕ್ಲಾಮ್ಪ್ಸಿಯಾ ದ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
17	ಗರ್ಭಧಾರಣೆಯ ಕಾಲದಲ್ಲಿ ರಕ್ತದ ಒತ್ತಡದ ವಿಕಾರದ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
18	ಗರ್ಭವತಿ ಕಾಲದಲ್ಲಿ ಮಧುಮೇಹ ವಿಕಾರದ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
19	ರಕ್ತಕ್ಷಯದ ವಿಕಾರದ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
20	ಯಾವುದೇ ಸಂಸರ್ಗದ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
21	ಮಳಮಳಸುವುದು ಹಾಗೂ ವಾಂತಿ ಆಗುವ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
22	ಗರ್ಭಾವಸ್ಥೆಯಲ್ಲಿ ರಕ್ತಸ್ರಾವದ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
23	ಸಾಮಾನ್ಯ ಯೋನಿಮೂಲಕ ಪ್ರಸೂತಿಯಾದ ಇತಿಹಾಸ 1) ಒಂದೂ ಇಲ್ಲ 2) ಒಂದು 3) ಎರಡು 4) ಮೂರು ಹಾಗೂ ಹೆಚ್ಚು		
24	ಸಹಾಯ ಪಡೆದುಕೊಂಡು ಯೋನಿಮೂಲಕ ಪ್ರಸೂತಿಯಾದ ಇತಿಹಾಸ 1) ಒಂದೂ ಇಲ್ಲ 2) ಒಂದು 3) ಎರಡು 4) ಮೂರು ಹಾಗೂ ಹೆಚ್ಚು		
25	ಶಸ್ತ್ರಕ್ರಿಯೆ ಮೂಲಕ (ಸೀಝೆರಿಯನ್) ಆದ ಪ್ರಸೂತಿಯ ಇತಿಹಾಸ 1) ಒಂದೂ ಇಲ್ಲ 2) ಒಂದು 3) ಎರಡು 4) ಮೂರು ಹಾಗೂ ಹೆಚ್ಚು		
26	ಗರ್ಭಾವಸ್ಥೆನಂತರ ಆದ ರಕ್ತಸ್ರಾವದ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
27	ಮೃತಾವಸ್ಥೆಯಲ್ಲಿ ಭ್ರೂಣಜನಿಸಿದ ಇತಿಹಾಸ 1) ಒಂದೂ ಇಲ್ಲ 2) ಒಂದು 3) ಎರಡು 4) ಮೂರು ಹಾಗೂ ಹೆಚ್ಚು		

28	ಕಡಿಮೆ ತೂಕದ ಭ್ರೂಣಜನ್ಮಕ್ಕೆ ಬಂಧ ಇತಿಹಾಸ 1) ಒಂದೂ ಇಲ್ಲ 2) ಒಂದು 3) ಎರಡು 4) ಮೂರು ಹಾಗೂ ಹೆಚ್ಚು		
29	ಅವಳ-ಜವಳ ಜನ್ಮಕ್ಕೆ ಬಂದ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
30	ಅವಧಿ ಪೂರ್ವ ಪ್ರಸೂತಿಯಾದ ಇತಿಹಾಸ 1) ಒಂದೂ ಇಲ್ಲ 2) ಒಂದು 3) ಎರಡು 4) ಮೂರು ಹಾಗೂ ಹೆಚ್ಚು		
31	ಪ್ರಸೂತಿಯಾದನಂತರ ಮುಂದೆ ಭ್ರೂಣ ಮೃತ್ಯುಹೊಂದಿದ ಇತಿಹಾಸ 1) ಒಂದೂ ಇಲ್ಲ 2) ಒಂದು 3) ಎರಡು 4) ಮೂರು ಹಾಗೂ ಹೆಚ್ಚು		
32	ಪ್ರಸೂತಿನಂತರ ಜನ್ಮಜಾತ ವ್ಯಂಗವಾಗಿರುವ ಭ್ರೂಣದ ಇತಿಹಾಸ 1)ಹೌದು 2) ಇಲ್ಲ		
ಸಧ್ಯದ ಗರ್ಭಧಾರಣೆಯ ಸಂಬಂಧ ವಿವರ			
33	ಎಲ್.ಎಮ್.ಪಿ. (LMP) ತಾರೀಖ		
34	ಈ.ಡಿ.ಡಿ (EDD) ತಾರೀಖ		
35	ಜನ್ಮದ ಪ್ರಕಾರ 1) ಸಾಮಾನ್ಯ 2) ಸಹಾಯ ಪಡೆದು ಆದ ಪ್ರಸೂತಿ		
36	ಗರ್ಭಾವಸ್ಥೆ		
37	ವರ್ಗ		
38	ವಯಸ್ಸು ಆರ್ಡರ (ಪ್ರಸೂತಿಯ ಪರಿಸ್ಥಿತಿ)		
39	ಸಧ್ಯದ ಆಗು ಹಿಂದಿನ ಗರ್ಭಾಧಾರಣೆಯ ಅಂತರ		
40	ಎ.ಎನ್.ಸಿ. ಗಾಗಿ ಭೇಟಿನೀಡಿ (ವ್ಹಿಜಿಟ) 1) ಒಂದು 2) ಎರಡು 3) ಮೂರು 4) ನಾಲ್ಕು 5) ಒಮ್ಮೆಯೂ ಇಲ್ಲ		
41	ಪ್ರಸೂತಿಯಾದ ನಂತರ ರಕ್ತಸ್ರಾವ 1)ಹೌದು 2) ಇಲ್ಲ		
42	ಸಮಯದ ಮೊದಲು ಒಳಗಿನ ತ್ವಚೆ ಹರಿಯುವದು 1)ಹೌದು 2) ಇಲ್ಲ		
43	ಪ್ರಿ- ಎಕ್ಲಾಮ್ಪ್ಸಿಯಾ 1)ಹೌದು 2) ಇಲ್ಲ		
44	ಗರ್ಭಾವಸ್ಥೆಯಲ್ಲಿ ರಕ್ತದ ಒತ್ತಡದ ವಿಕಾರ 1)ಹೌದು 2) ಇಲ್ಲ		
45	ಗರ್ಭಾವಸ್ಥೆಯಲ್ಲಿ ಮಧುಮೇಹದ ವಿಕಾರ 1)ಹೌದು 2) ಇಲ್ಲ		
46	ಕಾಮಿನಿ 1)ಹೌದು 2) ಇಲ್ಲ		
47	ರಕ್ತದ ಒತ್ತಡ 1)ಹೌದು 2) ಇಲ್ಲ		
48	ಕಂಠಗ್ರಂಥಿಯ (ಥಾಯರಾಯಿಡ) ವಿಕಾರ 1)ಹೌದು 2) ಇಲ್ಲ		
49	ನಿಮಗೆ ಗಭಾವಸ್ಥೆಯಲ್ಲಿ ಇತರ ಯಾವುದೇ ರೋಗ ಬಂದಿವೆಯೋ ಹೇಗೆ ? 1)ಹೌದು (ವಿವರ ನೀಡಿ) 2) ಇಲ್ಲ		
50	ರಕ್ತದ ಗುಂಪು : 1) A^+ 2) B^+ 3) B 4) AB^+ 5) A^- 6) B^- 7) O^- 8) AB^-		
51	ಎಚ್-ಬಿ (HB) ಗ್ರಾಮ %		
52	ಫಾಲಿಕ ಅಸಿಡ ಅಥವಾ ಪೂರೈಕೆ 1)ಹೌದು 2) ಇಲ್ಲ		
53	ಕ್ಯಾಲ್ಶಿಯಮ ಪೂರೈಕೆ 1)ಹೌದು 2) ಇಲ್ಲ		
54	ಇತರ ಯಾವುದೇ ಔಷಧಗಳು (ವಿವರ ನೀಡಿ)		

ಭ್ರೂಣದ ವಿವಿಧತೆ ಹಾಗೂ ಪ್ರಸೂತಿ ಬಗ್ಗೆ ಮಾಹಿತಿ			
ಅ.ನಂ.	ಸಾಂಕೇತಿಕ ಶಬ್ದಸಹ ಬದಲಾಗುವ ಮಾಹಿತಿ	ಪ್ರತಿಕ್ರಿಯೆ ಸಾಂಕೇತಿಕ ಶಬ್ದಸಹ	ಶರಾ
55	ಪ್ರಸೂತಿಯ ತಾರೀಖು		
56	ಪ್ರಸೂತಿ ವೇಳೆಗೆ ಗರ್ಭಧಾರಣೆಯ ಒಟ್ಟು ವಾರಗಳು		
57	ಪ್ರಸೂತಿಯ ಪ್ರಕಾರ 1) ಯೋನಿಮಾರ್ಗ ಸಾಮಾನ್ಯವಾಗಿ 2) ಸಹಾಯ ಪಡೆದು 3) ಶಸ್ತ್ರಕ್ರಿಯೆಯಿಂದ		
58	ಭ್ರೂಣದ ಅವಸ್ಥೆ 1) ವೆರ್ಟೆಕ್ಸ 2) ಬೀಚ 3)ಓಬ್ಲಿಕ 4) ಇತರ		
59	ಅಂಗ : 1) ಪುರುಷ 2) ಸ್ತ್ರೀ		
60	ಮಾಸದ ಹೆರಿಗೆಯ ಪ್ರಕಾರ 1) ಸಾಮಾನ್ಯ 2) ರಿಟೇಡ್		
61	ಮಾಸದ (ವಾರದ) ತೂಕ (ಗ್ರ್ಯಾಮ್ಸ)		
62	ಮಾಸದ ಘನಫಲ (ಮಿಲಿ)		
63	ಮಾಸದ ಪೃಷ್ಠಭಾಗ (ಸೆ.ಮಿ.^{2})		
64	ಆನ್ಮತಃ ಇರುವ ತೂಕ (ಗ್ರ್ಯಾಮ)		
65	ಉದ್ದ (ಸೆ.ಮಿ)		
66	ತಲೆಯ ಸುತ್ತಳತೆ (ಸೆ.ಮಿ)		
67	ಹೊಟ್ಟೆಯ ಸುತ್ತಳತೆ (ಸೆ.ಮಿ)		
68	ಹುಟ್ಟಿನಿಂದ ಇರುವ ವಿಕೃತಿ ಅಥವಾ ಅಸಂಬದ್ಧವಾದ		
69	ಭ್ರೂಣದಲ್ಲಿ ಜನ್ಮತಃ ಇರುವ ರೋಗ (ಡಾಕ್ಟರರ ಶರಾ)		
ಹಿಸ್ಟಾಲಾಜಿಕಲ ಮತ್ತು ಇಮ್ಯುನೊಹಿಸ್ಟೊಕೆಮಿಕಲ್ ವಿವಿಧತೆಯ ಮಾಹೀತಿ			
70	ಮಾಸದ ಪೃಷ್ಠಭಾಗ		
71	ರಕ್ತವಾಹಿನಿಯ ಬೆಳವಣಿಗೆ ಅಂಶ ಎ ದ ಪ್ರಮಾಣ		
72	ರಕ್ತನಾಳ /ವಿಲ್ಯೆ		

ठराविक पध्दतीनुसार (Proforma)

प्रकल्पाचा मंथळा : इम्यूनोहिस्टोकेमीकल इवॅल्यूएशन ऑफ वॅस्कूलर एन्डोथीलाईल ग्रोथ फॅक्टर इन ह्यूमन प्लॅसेंटा ॲन्ड इटस रोल इन ग्रोथ ऑफ प्लॅसेंटा ॲन्ड फेटस

सि. नं.

प्रवेश ता.
रजिस्ट्रेशन नं.

सामाजिक लोकसंख्येचा शास्त्रशुध्द अभ्यासाची माहिती :

अ, नं.	सांकेतिक शब्दासह बदलू शकणारी माहिती	प्रतिक्रिया, सांकेतिक शब्दासह	शेरा
1	नांव, पत्ता, मोबाईल नं.		
2	वय		
5	धर्म : 1) हिंदू 2) मुस्लीम 3) खिश्चन 4) जैन 5) इतर		
6	प्रतीविषयक संबंध : 1) रक्ताचे नाते असलेला 2) रक्ताचे नाते नसलेला		

जीवनशैली आणि संसर्गाची शक्यता :

अ, नं.	सांकेतिक शब्दासह बदलू शकणारी माहिती	प्रतिक्रिया, सांकेतिक शब्दासह	शेरा
7	सवयी 1) तंबाखू चघळणे 2) मद्य 3) अंमली पदार्थाचे व्यसन		
8	व्यवसाय : 1) शेतमजूर 2) मशागत करणारा 3) घरगुती कार्य 4) नोकर 5) मालक 6) काम न करणारा 7) भाडे घेणारा (घरमालक) 8) विद्यार्थी 9) काम न करणारा		
9	खाण्याच्या सवयी 1) शाकाहारी अ) दुग्ध पदार्थ ब) Ova शाकाहारी 2) मांसाहारी 3) प्राण्यापासून तयार झालेले		

आधीच्या गरोदर पणाचा इतिहास :

अ. नं.	सांकेतिक शब्दासह बदलू शकणारी माहिती	प्रतिक्रिया, सांकेतिक शब्दासह	शेरा
10	लग्नाच्या वेळचे वय (वर्षामध्ये)		
11	पहिल्या गरोदरपणावेळचे वय		
12	पूर्वीच्या गरोदरपणामध्ये असलेले जन्माचे अंतर		
13	गर्भनिरोधकांचा वापर 1) होय 2) नाही		
14	आपोआप गर्भपात झालेल्या घटना : 1) एकही नाही 2) एक 3) दोन 4) तीन आणि आधिक		
15	घडवून आणलेल्या गर्भपाताच्या घटना 1) एकही नाही 2) एक 3) दोन तीन आणि आधिक		
16	प्री–एक्लॅम्प्सीया चा इतिहास 1) होय 2) नाही		
17	गर्भवती काळामध्ये असलेल्या रक्तदाबाच्या विकाराचा इतिहास 1) होय 2) नाही		
18	गर्भवती काळामध्ये असलेल्या मधूमेहाच्या विकाराचा इतिहास 1) होय 2) नाही		
19	रक्तक्षयाच्या विकाराचा इतिहास 1) होय 2) नाही		
20	कोणत्याही संसर्गाचा इतिहास 1) होय 2) नाही		
21	मळमळ आणि उलटी होण्याचा इतिहास 1) होय 2) नाही		
22	गरोदर पणातील रक्तस्त्रावाचा इतिहास 1) होय 2) नाही		
23	सामान्य योनीद्वारे प्रसुती झाल्याचा इतिहास 1) एकही नाही 2) एक 3) दोन 4) तीन आणि आधिक		
24	सहायता घेऊन योनीद्वारा प्रसुती झाल्याचा इतिहास : 1) एकही नाही 2) एक 3) दोन 4) तीन आणि आधिक		
25	शस्त्रक्रियेद्वारा (सिझरिन) झालेल्या प्रसुतीचा इतिहास 1) एकही नाही 2) एक 3) दोन 4) तीन आणि आधिक		
26	गरोदर पणानंतर झालेल्या रक्तस्त्रावाचा इतिहास 1) होय 2) नाही		
27	मृतावस्थेमध्ये अर्भक जन्मल्याचा इतिहास 1) एकही नाही 2) एक 3) दोन 4) तीन आणि आधिक		

28	कमी वजनाचे अर्भक जन्माला येण्याचा इतिहास 1) एकही नाही 2) एक 3) दोन 4) तीन आणि आधिक		
29	जुळे जन्माला येण्याचा इतिहास 1) होय 2) नाही		
30	वेळेआधी प्रसुती होण्याचा इतिहास 1) एकही नाही 2) एक 3) दोन 4) तीन आणि आधिक		
31	प्रसुती झाल्यानंतर पुढे अर्भकाचा मृत्यू होण्याचा इतिहास 1) एकही नाही 2) एक 3) दोन 4) तीन आणि आधिक		
32	प्रसुतीनंतर जन्मजात व्यंग असलेल्या अर्भकाचा इतिहास 1) होय 2) नाही		
सध्याचा गरोदरपणाशी संबंधीत माहिती			
33	एल एम पी (LMP) तारीख		
34	ई डी. डी. (EDD) तारीख		
35	जन्माचा प्रकार 1) सामान्य 2) सहायता करुन केलेली प्रसुती		
36	गरोदर पणा		
37	दर्जा		
38	वय ऑर्डर (प्रसुतीची परिस्थिती)		
39	सध्याच्या आणि पूर्विच्या गरोदरपणातील अंतर		
40	ए.एन.सी साठी भेटा (विजिट) 1) एक 2) दोन 3) तीन 4) चार 5) एकदाही नाही		
41	गरोदरपणानंतर रक्तस्त्राव 1) होय 2) नाही		
42	वेळेच्या आधी अंतत्वचा फाटणे 1) होय 2) नाही		
43	प्री–एक्लॅम्प्सीचा 1) होय 2) नाही		
44	गरोदरपणातील रक्तदाबाचा विकार 1) होय 2) नाही		
45	गरोदरपणातील मधूमेहाचा विकार 1) होय 2) नाही		
46	कावीळ 1) होय 2) नाही		
47	रक्तदाब 1) होय 2) नाही		
48	कंठग्रंथीचा (थॉयराईड) विकार 1) होय 2) नाही		
49	तुम्हाला गरोदर अवस्थे मध्ये इतर कोणतेही आजार झाले आहेत काय ? 1) होय (तपशील द्या 2) नाही		
50	रक्तगट 1) A^+ 2) B^+ 3) B 4) AB^+ 5) A^- 6) B^- 7) O^- 8) AB^-		
51	एच बी (H.B) ग्राम %		
52	फॅालीक आम्ल या पुरवठा 1) होय 2) नाही		

53	कॅल्शीयम पुरवठा 1) होय 2) नाही		
54	इतर कोणतेही औषधे (तपशील द्या)		
	फेटल विविधतेची आणि प्रसुतीसंबंधी माहिती		
अ. नं.	सांकेतिक शब्दासह बदलू शकणारी माहिती	प्रतिक्रिया, सांकेतिक शब्दासह	शेरा
55	प्रसुतीची तारीख		
56	प्रसुतीसमयी गर्भारपणाची एकूण आठवडे		
57	प्रसुतीचा प्रकार 1) योनीमार्गे सामान्यपणे 2) सहायता करुन 3) सिझरीयन सेक्शन		
58	फेटल अवस्था 1) वेर्टेक्स 2) बीच 3) ओब्लीक 4) इतर		
59	लिंग 1) पुरुष 2) स्त्री		
60	प्लॅसेंटल डिलीवरीचा प्रकार 1) सामान्य 2) रीटेनड		
61	प्लॅसेंटल (वार) चे वजन (ग्राम्न)		
62	प्लॅसेंटाचे घनफळ (मिली)		
63	प्लॅसेंटाच पृष्ठभाग (से.मी^{2})		
64	जन्मतः असलेले वजन (ग्राम)		
65	लांबी (सेंमी)		
66	डोक्याचा घेर (से. मी)		
67	पोटाचा घेर (से.मी)		
68	जन्मजात असलेली विकृती किंवा असंबध्द गोष्ट		
69	अर्भकामध्ये जन्मताच असलेले रोग (डॉक्टरचा शेरा)		
	हिस्टॅलॉजिकल आणि इम्यूनोहिस्टाकेमिकल विविधतेची माहिती		
70	प्लॅसेंटाचा पृष्ठभाग		
71	(वीईजीएफ) चे प्रमाण		
72	(Vesselsa) वेसेल्स/विली (villi) प्लॅसेंटा		

ANNEXURE –I I – INFORMED CONSENT & PATIENT INFORMATION SHEET

KLE UNIVERSITY'S J.N. MEDICAL COLLEGE, NEHRU NAGAR , BELAGAVI

PARTICIPANTS INFORMATION SHEET

This Informed Consent Form is for women who admit for delivery and who we are inviting to participate in research on Vascular Endothelial Growth Factor-A in placenta.

The Title of the project: Immunohistochemical Evaluation of Vascular Endothelial Growth Factor-A (VEGF-A) in Human Placenta and Its Role in Growth of Placenta and Fetus.

Name of the Investigator: Mrs.Vanitha

Name of Organization: Department of Anatomy,
KLE University's JN Medical College,
Belagavi.

Introduction: I am Vanitha Full time Ph.D. scholar in the department of Anatomy JNMC Belagavi. We are doing research on Vascular endothelial growth factor-A in the placenta. The placenta is connecting organ between mother and developing fetus. Vascular endothelial growth factor-A is present in the placenta helps in the formation of the blood vessel and in the development of the placenta. Defects in development of placenta lead to the alteration in the development of the fetus. This study has been taken to see Vascular endothelial growth factor-A expression in the placenta which helps in vascular development thereby fetal growth.

I am giving you information and inviting you to be part of this research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them to me.

Purpose of the research: Growth factors have been shown to play an important role in placental development and their imbalance is associated with the placental malformation. Vascular endothelial growth factor-A helps in vascular development, thereby increases nutrient transfer from mother to fetus, which is necessary for growth. This study focuses on its expression and level of Vascular endothelial growth factor-A, which may help to understand its normal function and can able to explain the reasons behind the complications in pregnancy.

Voluntary Participation: Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this hospital will continue and nothing will change.

Procedure: In this study, we will collect placenta after the delivery. Placenta is a single tissue, which is expelled after delivery. It is not important to your body after the delivery and it will be discarded. We are using this tissue for our study which is going to be discarded. If you give this tissue for our study, we will take weight, volume and surface area of placenta and will section the placenta into small pieces to study vascular endothelial growth factor-A which helps in the development of vessels and the placenta. So that we can understand its function and the reasons behind the complications during pregnancy. We will also take measurements of your baby like weight, height and circumference with the help of nursing staff in the ward. You will also have to write answers to questions which are written in a form related to information on present pregnancy, previous pregnancy, health treatments which are undergone.

Duration of the procedure: The entire procedure may take about 15 minutes if you agree to participate.

Risk: In this study there is no risk involved, as this study is purely based on a questionnaire which you are supposed to answer and there is no intervention done in any form.

Benefits: Your participation is likely to be helpful for us to find the answer to the research question. There may not be any benefit to the society at this stage of the research, but future generations are likely to benefit.

Reimbursements: No incentives will be given to you for the participating in this study. There will be no additional cost borne by you in any form for participating in this study. The cost of the study will be borne by the researcher.

Confidentiality: The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone.

Sharing the Results: The knowledge that we get from doing this research will be presented in front of research committee before it is made widely available to the public. Confidential information will not be shared. After the presentation, we will publish the results in order that other interested people may learn from our research.

Withdrawal rights: You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will be respected.

Who to Contact:

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following,

1. Mrs. Vanitha, Mob no: 8095600731
2. Dr. Daksha Dixit, Professor, Department of Anatomy, KLE University's JN Medical College, Belagavi. Mob no: 9036836799
3. Dr. Anil Hogade, Chairman, Ethical Committee KLE University, Belagavi. 0831-2473777 Exten.4064/4095

Legal Rights: By signing this consent form, we are not waiving any of your legal rights.

Consent Form

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of Participant _____

Signature of participant _____

Date:_____
Day/month/year

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness_____ AND Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher:

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the

individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Name of Researcher _____

Signature of Researcher _____

Date _____

Day/month/year

ಕೆ.ಎಲ್.ಇ. ಸೊಸಾಯಿಟಿ ಜೆ.ಎನ್.ವೈದ್ಯಕೀಯ ಕಾಲೇಜ್, ನೆಹರು ನಗರ, ಬೆಳಗಾವಿ.

ಬಾಗವಹಿಸುವವರ ಮಾಹಿತಿ ಪತ್ರ.

ಈ ಮಾಹಿತಿಪೂರ್ಣ ಸಮ್ಮತಿ ಪತ್ರ ಯಾರು ಪ್ರಸೂತಿಗಾಗಿ ಪ್ರವೇಶ ತೆಗೆದುಕೊಂಡಿದ್ದಾರೆ ಆ ಮಹಿಳೆಯರಿಗಾಗಿ ಇದೆ. ಹಾಗೂ ಅವರಿಗೆ ನಾವು ರಕ್ತವಾಹಿನಿ ಬೆಳವಣಿಗೆಯ ಅಂಶ–ಎ ಮೇಲೆ ಆಗುವ ಸಂಶೋದನೆಯಲ್ಲಿ ಸಹಭಾಗಿಯಾಗುವ ಬಗ್ಗೆ ಆಮಂತ್ರಿಸಲಾಗುತ್ತಿದೆ.

ಪ್ರಕಲ್ಪದ ಶಿರ್ಷಿಕೆ : ಮಾಸದಲ್ಲಿನ ರಕ್ತವಾಹಿನಿಯ ಬೆಳವಣಿಗೆಯ ಅಂಶ–ಎ ಅದರ ಇಮ್ಯುನೊಹಿಸ್ಟೋಕೆಮಿಕಲ್ ಮೌಲ್ಯಮಾಪನ ಮತ್ತು ಮಾಸು ಹಾಗೂ ಭ್ರೂಣದ ಬೆಳವಣಿಗೆಯಲ್ಲಿ ಅದರ ಮಹತ್ವ.

ಸಂಶೋಧಕರ ಹೆಸರು : ಶ್ರೀಮತಿ ವನಿತಾ
ಸಂಸ್ಥೆಯ ಹೆಸರು : ಶರೀರ ರಚನೆಯ ಶಾಸ್ತ್ರ ವಿಭಾಗ ಕೆ.ಎಲ್.ಇ.ಜೆ.ಎನ್. ಮೆಡಿಕಲ್ ಕಾಲೇಜು
ಬೆಳಗಾವಿ ಮಹಿತಿಪೂರ್ಣ ಸಮ್ಮತಿ ಪತ್ರದ ಎರಡು ಭಾಗ ಇದೆ.

ಮಾಹಿತಿ ಪತ್ರ :

ಪರಿಚಯ : ನಾನು ವನಿತಾ ಇದ್ದೇನೆ, ನಾನು ಜೆ.ಎನ್.ಎಮ್.ಸಿ. ಬೆಳಗಾವಿಯ ಶರೀರ ರಚನೆ ಶಾಸ್ತ್ರ ವಿಭಾಗಲದಲ್ಲಿ ಸಂಶೋಧನೆಯ ಪೂರ್ಣ ವೇಳೆ ವಿದ್ಯಾರ್ಥಿನಿಯಾಗಿದ್ದೇನೆ. ನಾನು ಮಾಸುವಿನ ರಕ್ತವಾಹಿನಿಯ ಬೆಳವಣಿಯ ಅಂಶ–ಎ ಮೇಲೆ ಸಂಶೋಧನೆ ಮಾಡುತ್ತಿದ್ದೇವೆ.

ಮಾಸುವು ತಾಯಿ ಮತ್ತು ಬೆಳೆಯುತ್ತಿರುವ ಭ್ರೂಣಕ್ಕೆ ಸಂಪರ್ಕ ಕಲ್ಪಿಸುತ್ತದೆ ಹಾಗೂ ಬೆಳವಣಿಗೆಗೆ ಬೇಕಾಗುವ ಪೋಷಣೆಗಳನ್ನು ಸಾಗಿಸುತ್ತದೆ ರಕ್ತವಾಹಿನಿಯ ಅಂಶ ಮಾಸುವಿನಲ್ಲಿ ಇರುತ್ತದೆ ಹಾಗೂ ಇದು ರಕ್ತವಾಹಿನಿಯ ಬೆಳವಣಿಗೆ ಮತ್ತು ಮಾಸುವಿನ ಬೆಳವಣಿಗೆಗೆ ಸಹಾಯವಾಗುತ್ತದೆ. ಸರಿಯಾದ ಭ್ರೂಣದ ಬೆಳವಣಿಗೆಗೆ, ಮಾಸುವಿನ ಬೆಣವಣಿಗೆ ಮತ್ತು ಅರೆ ಕಾರ್ಯವೈಖರಿ ಪ್ರಮುಖ ಅಂಶವಾಗಿರುತ್ತದೆ. ಅದರ ಕಾರ್ಯವೈಖರಿ ಧೋಷಗಳು ಭ್ರೂಣದ ಬೆಳವಣಿಗೆಯಲ್ಲಿ ಅದರ ಮಹತ್ವ ತಿಳಿಯಲು ಪ್ರಯತ್ನಿಸುತ್ತಿದ್ದೇವೆ ಹಾಗೂ ನಿಮಗೆ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಆಹ್ವಾನಿಸುತ್ತೇವೆ.

ಇಲ್ಲಿ ನಿಮಗೆ ಅರ್ಥವಾಗದ ಕೆಲವು ಪದಗಳು ಇರಬಹುದು. ಅವು ನಿಮಗೆ ತಿಳಿಯುವುದಿಲ್ಲ ಆಗ ನಾನು ನಿಮಗೆ ಮಾಹಿತಿ ಹೇಳುತ್ತಿರುವಾಗ ದಯಮಾಡಿ ನಿಲ್ಲಿರಿ. ಮತ್ತು ನಾನು ನಿಮಗೆ ತಿಳಿಸಿ ಹೇಳಲು ಸಾಧ್ಯ ಒಂದು ವೇಳೆ ನಿಮಗೆ ಈ ಮಾಹಿತಿ ನಂತರ ಪ್ರಶ್ನೆ ಕೇಳುವುದಾದಲ್ಲಿ ನೀವು ನನ್ನನ್ನು ಕೇಳಬಹುದು.

ಸಂಶೋದನೆಯ ಉದ್ದೇಶ : ಬೆಳವಣಿಗಯ ಅಂಶ ಮಾಸದ ಬೆಳವಣಿಗೆಯಲ್ಲಿ ಮಹತ್ವದ ಭೂಮಿಕೆ ಹೊಂದಿದ್ದು ಅದರ ಅನಿಯಂತ್ರಣ ಮಾಸದ ರೋಗ ವಿಜ್ಞಾನಕ್ಕೆ ಸಂಬಂಧಿತವಾಗಿರುತ್ತದೆ. ರಕ್ತವಾಹಿನಿಯ ಅಂಶ–ಎ ಇದು ರಕ್ತವಾಹಿನಿ ವಿಕಾಸದಲ್ಲಿ ಸಹಾಯ ಮಾಡುತ್ತದೆ. ಕಾರಣ ತಾಯಿಯಿಂದಯ ಭ್ರೂಣಕ್ಕೆ ಬರುವ ಪೌಷ್ಟಿಕ ಪದಾರ್ಥಗಳ ಪ್ರಮಾಣ ಹೆಚ್ಚಿಸುತ್ತದೆ. ಮತ್ತು ಭ್ರೂಣದ ಬೆಳವಣಿಗೆ ಅನಶ್ಯವಾಗಿದೆ.

ಈ ಅಭ್ಯಾಸ ಅದರ ಕಾರ್ಯದ ಮೆಲೆ ಹಾಗೂ ಅದರ ತಳಹದಿಯಲ್ಲಿ ಗಮನ ಕೇಂದ್ರಿಕೃತವಾಗುತ್ತದೆ. ಕಾರಣ ಸಾಮಾನ್ಯ ಶರೀರ ವಿಜ್ಞಾನ ಶಾಸ್ತ್ರ ತಿಳಿದುಕೊಳ್ಳಲು ಸಹಾಯವಾಗುತ್ತದೆ. ಹಾಗೂ ಗರ್ಭಾವಸ್ಥೆಯಲ್ಲಿಯ ಎಲ್ಲಾ ಸಮಸ್ಯೆಗಳನ್ನು ಶೋಧಿಸಲು ಸಹಾಯ ಮಾಡುತ್ತದೆ.

ಐಚ್ಛಿಕ ಸಹಭಾಗತ್ವ : (ಸ್ವ ಇಚ್ಚೆಯಿಂದ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ) :

ನೀವು ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಸಹಭಾಗಿಯಾಗಲು ಸ್ವತಂತ್ರರಿರುತ್ತೀರಿ ಪೂರ್ಣವಾಗಿ ನಿಮ್ಮ ಹಟೋಟಿಯಲ್ಲಿರುತ್ತದೆ. ಭಾಗವಹಿಸುವದು ಅಥವಾ ಬಿಡುವುದು ನಿಮ್ಮ ಇಚ್ಚೆ ಹಾಗೂ ಆಸ್ಪತ್ರೆಯಲ್ಲಿಯ ಸೇವೆಯ ಮೇಲೆ ತಮ್ಮ ನಿರ್ಣಯದಿಂದ ಯಾವ ಬದಲಾವಣೆಯೂ ಅಗುವುದಿಲ್ಲ.

ಕಾರ್ಯಪದ್ಧತಿ :

ಈ ಅಭ್ಯಾಸದಲ್ಲಿ ನಾವು ಮಾಸವನ್ನು ಪ್ರಸವದ ನಂತರ ತೆಗೆದುಕೊಳ್ಳುತ್ತೇವೆ. ಮಾಸವು ಒಂದು ಅಂಗಾಂಸ ಇದು ಪ್ರಸವದ ನಂತರ ಗರ್ಭಕೋಶದಿಂದ ಹೊರದೂಡಲ್ಪಡುತ್ತದೆ. ಎಲ್ಲಾ ಪ್ರಸವದ ಸಂದರ್ಭಗಳಲ್ಲಿ ಇದನ್ನು ಪ್ರಸವದ ನಂತರ ಎಸೆಯಲಾಗುತ್ತದೆ ಮತ್ತು ಇದು ಪ್ರಸವದ ನಂತರ ನಿಮ್ಮ ದೇಹಕ್ಕೆ ಯಾವದೇ ಉಪಯೋಗಕ್ಕೆ ಬರುವುದಿಲ್ಲ. ನೀವು ಈ ಅಂಗಾಂಶವನ್ನು ನಮ್ಮ ಅಭ್ಯಾಸಕ್ಕೆ ಕೊಡಲು ಇಚ್ಚಿಸದರೆ ನಾವು ಇದನ್ನು ತೆಗೆದುಕೊಳ್ಳು ಬಯಸುತ್ತೇವೆ. ಈ ಅಂಗಾಂಶವನ್ನು ನಾವು ಪಡೆಹಿಡಿದುಕೊಂಡು ಅದರ ತೂಕ, ಘತ ಅಳತೆ, ಮತ್ತು ಮೇಲ್ಮೈ ಅಳತೆಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುತ್ತೇವೆ. ಹಾಗೂ ಅದನ್ನು ಸಣ್ಣ ತುಂಡುಗಳಾಗಿ ಕತ್ತರಿಸಿ, ರಕ್ತವಾಹಿಯು ಅಂಶವನ್ನು ತಿಳಿಯಲು ಬಳಸುತ್ತೇವೆ. ಇದರಿಂದ ನಮಗೆ ಮಾಸುವಿನಲ್ಲಿನ ರಕ್ತವಾಹಿನಿಯ ಅಂಶ ಮತ್ತು ಅದರ ಕೆಲಸ ಹಾಗೆಯೇ ಮಾಸುವಿನ ಬೆಳವಣಿಗೆಯಲ್ಲಿ ಅದರ ಮಹತ್ವ ಎನು ಎಂಬ ತಿಳಿಯಲು ಸಹಾಯವಾಗುತ್ತದೆ. ಹಾಗೂ ಗರ್ಭಾವಸ್ಥೆಯಲ್ಲಿ ಆದ ಸಮಸ್ಯಗಳು ತಿಳಿಯಲು ಸಾದ್ಯವಾಗುತ್ತದೆ. ಇದರ ಜೊತೆಗೆ ನಾವು ನಿಮ್ಮ ಮಗುವಿನ ತೂಕ, ಎತ್ತರ, ಮತ್ತು ಸುತ್ತಳತೆಯನ್ನು ಒಬ್ಬ ದಾದಿಯ ಸಹಾಯದಿಂದ ತೆಗೆದುಕೊಳ್ಳತ್ತೇವೆ.

ಹಾಗೆಯೇ ನಾವು ನಿಮಗೆ ಸದ್ಯದ ಗರ್ಭಾವಸ್ಥೆ, ಹಿಂದಿನ ಗರ್ಭಾವಣೆ ಮತ್ತು ಆರೋಗ್ಯ ಉಪಚಾರದ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಬರೆದಿಟ್ಟ ಮಾಹಿತಿ ಪತ್ರವನ್ನು ಕೊಡುತ್ತೇವೆ ಮತ್ತು ನೀವು ಅದಕ್ಕೆ ಉತ್ತರಿಸಬೇಕಾಗುತ್ತದೆ.

ಕಾರ್ಯ ಪದ್ಧತಿಯ ಅವಧಿ :

ತಾವು ಇದರಲ್ಲಿ ಸಹಭಾಗಿಯಾಗಲು ಸಮ್ಮತಿ ನೀಡಿದಲ್ಲಿ ಸಂಪೂರ್ಣ ಕಾರ್ಯ ಪದ್ಧತಿಗೆ 15 ನಿಮಿಷಗಳು ತಗಲುತ್ತವೆ.

ಅಪಾಯ : ಈ ಅಭ್ಯಾಸದಲ್ಲಿ ಯಾವುದೇ ಅಪಾಯವಿಲ್ಲ ಕಾರಣ ಇದು ಪ್ರಶ್ನಾಧಾರಿತವಾಗಿದೆ. ಅವುಗಳಿಗೆ ನೀವು ಉತ್ತರ ನೀಡಬೇಕಾಗುತ್ತದೆ. ಇದರಲ್ಲಿ ಯಾವುದೇ ಮಧ್ಯಸ್ಥಿಕೆ ಮಾಡಲಾಗುವುದಿಲ್ಲ.

ಲಾಭ : ಸಂಶೋಧನೆಯಲ್ಲಿ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುವುದಕ್ಕಾಗಿ ನಿಮ್ಮ ಸಹಭಾಗಿತ್ವ ನಮಗೆ ಉಪಯೋಗವಾಗುತ್ತದೆ. ಆದರೆ ತಾವು ತಮ್ಮ ಸಹಬಾಗಿಯಾದ ಕಾರಣ ನಮಗೆ ಯಾವದೇ ಲಾಭ ದೊರೆಯುವುದಿಲ್ಲ ಆದರೆ ಭಾವಿ ಪೀಲಿಗೆಗೆ ಈ ಅಭ್ಯಾಸದ ಲಾಭ ದೊರೆಯುತ್ತದೆ.

ಪರಿಹಾರ : ಈ ಅಭ್ಯಾಸದಲ್ಲಿ ಸಹಭಾಗಿಯಾಗುವುದಕ್ಕಾಗಿ ನಿಮಗೆ ಯಾವುದೇ ಮೊಬದಲೆ ದೊರೆಯುವುದಿಲ್ಲ. ಹಾಗೂ ಇದರಲ್ಲಿ ಭಾಗ ವಹಿಸುವುದರಿಂದ ನಿಮಗೆ ಬೇರೆ ಖರ್ಚು ಸಹನೆ ಮಾಡುವ ಸಮಸ್ಯೆ ಬರುವುದಿಲ್ಲ ಈ ಅಭ್ಯಾಸದ ಖರ್ಚು ಸಂಪೂರ್ಣವಾಗಿ ಸಂಶೋಧಕರದು ಇರುತ್ತದೆ.

ಗೌಪ್ಯತೆ : ಈ ಸಂಶೋಧನೆಯ ಪ್ರಕಲ್ಪದಿಂದ ಜಮೆಯಾದ ಮಾಹಿತಿ ಗೌಪ್ಯವಾಗಿಡಲಾಗುವುದು. ನಿಮ್ಮ ಕಡೆಯಿಂದ ತೆಗೆದುಕೊಂಡ ಮಾಹಿತಿ ಒಂದು ಕಡೆಗೆ ಸಂಗ್ರಹಿಸಿ ಇಡಲಾಗುತ್ತದೆ. ಹಾಗೂ ಅದನ್ನು ಕೇವಲ ಸಂಶೋಧಕರಿಗೆ ಮಾತ್ರ ನೋಡಲು ದೊರೆಯುತ್ತದೆ. ನಿಮ್ಮ ಬಗ್ಗೆ ಯಾವುದೇ ಮಾಹಿತಿ ಮೇಲೆ ನಿಮ್ಮ ಹೆಸರು ಇರುವುದಿಲ್ಲ. ಕೇವಲ ಸಂಖ್ಯೆ ಇರುತ್ತದೆ. ಕೇವಲ ಸಂಶೋಧಕರಿಗೆ ನಿಮ್ಮ ಸಂಖ್ಯೆ ಗೊತ್ತಿರುತ್ತದೆ. ನಾವು ಆ ಮಾಹಿತಿಯನ್ನು ಭದ್ರತೆಯಲ್ಲಿಡುತ್ತೇವೆ. ಹಾಗೂ ಯಾರಿಗೂ ಅದನ್ನು ತೋರಗೊಡುವುದಿಲ್ಲ. ಅಥವಾ ಯಾರಿಗೂ ಕೊಡಲಾಗುವುದಿಲ್ಲ.

ಪರಿಣಾಮದ ಬಳಕೆ : ಈ ಅಭ್ಯಾಸಕ್ರಮದಿಂದ ನಮಗೆ ಯಾವ ಮಹಿತಿ ದೊರೆಯುತ್ತದೆ ಅದನ್ನು ಜನತೆಗೆ ಕೊಡುವ ಸಲುವಾಗಿ ಸಂಶೋಧನೆ ಸಮಿತಿಯ ಮುಂದೆ ಇಡಲಾಗುವದು ಗೌಪ್ಯ ಮಾಹಿತಿ ಬಹಿರಂಗಪಡಿಸಲಾಗುದಿಲ್ಲ. ಸಂಶೋಧನೆ ಸಮಿತಿಯ ಎದುರಿಗೆ ಮಂಡಿಸಿದ ನಂತರ ನಾವು ಆ ಪರಿಣಾಮವನ್ನು ಪ್ರಸಿದ್ಧೀಸುತ್ತೇವೆ. ಆ ಕಾರಣ ಸಾರ್ವಜನಿಕರಿಗೆ ನಮ್ಮ ಸಂಶೋಧನೆಯಿಂದ ಅವಶ್ಯಕ ಮಾಹಿತಿ ದೊರೆಯುತ್ತದೆ.

ಸಂಶೋಧನೆಯಿಂದ ಹಿಂದೆ ಸರಿಯಲು ಅಧಿಕಾರ : ನಿಮಗೆ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಇಚ್ಚೆ ಇಲ್ಲದಿದ್ದಲ್ಲಿ ತಾವು ಬೇಕಾದಾಗ ಹಿಂಪಡೆಯಬಹುದು ಅದಕ್ಕೆ ತಾವು ಸ್ವತಂತ್ರರಿರುವಿರಿ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗ ತೆಗೆದುಕೊಳ್ಳವ ಯಾವುದೇ ವೇಳೆ ನೀವು ತಿರಸ್ಕರಿಸಬಹುದು. ಅದು ನಿಮ್ಮ ಇಚ್ಛೆಯಾಗಿರುತ್ತದೆ. ಹಾಗೂ ನಾವು ತಮ್ಮ ಅಧಿಕಾರವನ್ನು ಗೌರವಿಸುತ್ತೇವೆ.

ಯಾರನ್ನು ಸಂಪರ್ಕಿಸಬೇಕು :
ಒಂದು ವೇಳೆ ತಮಗೆ ಪ್ರಶ್ನೆ ಕೇಳುವುದಿದ್ದಲ್ಲಿ ತಾವು ಈ ಕೆಳಗಿನವರನ್ನು ಸಂಪರ್ಕಿಸಬಹುದು.
ಅಭ್ಯಾಸ ಶುರವಾದ ನಂತರವೂ ಸಹ ನಿಮಗೆ ಪ್ರಶ್ನೆ ಕೇಳುವುದಿದ್ದಲ್ಲಿ ಈ ಕೆಳಗಿನವರನ್ನು ಸಂಪರ್ಕಿಸಬಹುದು.

1) **ಶ್ರೀಮತಿ ವನಿತಾ, ಮೊಬೈಲ್ ನಂ. 8095600731.**

2) **ಡಾ : ದಕ್ಷಾ ದಿಕ್ಷೀತ್, ಶರೀರ ರಚನೆ ಶಾಸ್ತ್ರ ವಿಭಾಗ**
ಕೆ.ಎಲ್.ಇ. ಸೊಸೈಟಿಯ ಜೆ.ಎನ್.ಎಮ್.ಸಿ. ಕಾಲೇಜ್
ಬೆಳಗಾವಿ ಮೊಬೈಲ್ ನಂ. 936836799.

3) **ಡಾ. ಅನೀಲ ಹೋಗಡೆ, ಅಧ್ಯಕ್ಷ ನೈತಿಸ ಸಮಿತಿ**
ಕೆ.ಎಲ್.ಇ.ಯುನಿವರ್ಸಿಟಿ, ಬೆಳಗಾವಿ ಮೊಬೈಲ್ ನಂ. 0831–2473777.
ಎಕ್ಸಟೆನ್‌ಶನ್ 4064/4095.

ಕಾಯ್ದೇಬದ್ದ ಹಕ್ಕು :

ಈ ಸಮ್ಮಿತಿ ಪತ್ರಕ್ಕೆ ತಾವು ಹಸ್ತಾಕ್ಷರ ನೀಡಿದ್ದರ ಬಗ್ಗೆ ಕಾಯ್ದೆ ಪ್ರಕಾರ ತಮ್ಮ ಯಾವುದೇ ಹಕ್ಕಿನ ತೊಂದರೆಯಾಗುವುದಿಲ್ಲ.

ಸಮ್ಮತಿ ಪತ್ರ

ನಾನು ಸದರೀ ಮಾಹಿತಿಯನ್ನು ಓದಿದೆನು/ನನಗೆ ಓದಿ ಹೇಳಲಾಯಿತು ನನಗೆ ಸದರೀ ವಿಷಯದ ಬಗ್ಗೆ ಪ್ರಶ್ನಿಸಲು ಅವಕಾಶವಿದ್ದು ನಾನು ಕೇಳಿದ ಪ್ರಶ್ನೇಗಳಿಗೆ ಸಮರ್ಪಕ ಉತ್ತರ ನೀಡಿದ್ದಾರೆ ನಾನು ಸಂತೋಷದಿಂದ ಈ ಸಂಶೋಧನಾ ಅಭ್ಯಾಸ ಕ್ರಮದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಮ್ಮತಿಸಿದ್ದೇನೆ.

ಭಾಗವಹಿಸಿದವರ ಹೆಸರು : _____

ಸಹಿ : _____

ದಿನಾಂಕ : _____
ದಿವಸ/ತಿಂಗಳು/ವರ್ಷ

ಅನಕ್ಷರಸ್ಥರಿದ್ದಲ್ಲಿ :

ಪಾಲ್ಗೋಳ್ಳುವವರಿಗೆ ಸದರೀ ವಿಷಯದ ಬಗ್ಗೆ ಎಲ್ಲ ಮಾಹಿತಿ ವಿವರವಾಗಿ ಓದಿ ತಿಳಿಸಿದ್ದು A ಹಾಗೂ ಅವರು ಅದಕ್ಕೆ ಒಪ್ಪಿದ್ದು ಮತ್ತು ಸದರೀಯವರಿಗೆ ವಿಷಯದ ಬಗ್ಗೆ ಪ್ರಶ್ನಿಸಲು ಅವಕಾಶ ಇದ್ದು ಪಾಲ್ಗೋಳ್ಳುವವರು ಸ್ವತಂತ್ರವಾಗಿ ತಮ್ಮ ಸಮ್ಮತಿ ನೀಡಿದ್ದಕ್ಕೆ ನಾನು ಸಾಕ್ಷಿ ಇದ್ದೇನೆ.

ಸಾಕ್ಷಿದಾರನ ಹೆಸರು : _____ ಮತ್ತು ಎಡಗೈ ಹೆಬ್ಬಟ್ಟಿನ ಗುರುತು

ಸಾಕ್ಷಿದಾರನ ಸಹಿ : _____

ದಿನಾಂಕ : _____
ದಿವಸ/ತಿಂಗಳು/ವರ್ಷ

ಸಂಶೋಧನಾಕಾರನ ಹೇಳಿಕೆ :

ನಾನು ಸೂಕ್ಷ್ಮವಾಗಿ ಈ ಅಭ್ಯಾಸ ಕ್ರಮದಲ್ಲಿ ಭಾಗವಹಿಸಿದವರ ಮಾಹಿತಿ ವಿವರ ಓದಿಕೊಂಡಿದ್ದೇನೆ. ಹಾಗೂ ಇದರಲ್ಲಿ ಭಾಗವಹಿಸಿದವರು ಎಲ್ಲ ವಿವರ ತಿಳಿದುಕೊಂಡ ಬಗ್ಗೆ ನನಗೆ

ಖಾತ್ರಿಯಾಗಿದೆ. ಭಾಗವಹಿಸಿದವರಿಗೆ ಸದರೀ ವಿಷಯದ ಬಗ್ಗೆ ಪ್ರಶ್ನೆ ಕೇಳಲು ಅವಕಾಶಗಳಿದ್ದ ಬಗ್ಗೆ ನಾನು ಖಾತ್ರಿ ಪಡಿಸಿಕೊಂಡಿದ್ದೇನೆ ಹಾಗೂ ಅವರು ಕೇಳಿದ ಎಲ್ಲ ಪ್ರಶ್ನೆಗಳಿಗೆ ಸಮರ್ಪಕ ಉತ್ತರ ನೀಡಿದೆ.

ಹಾಗೂ ಸದರೀಯವರಿಗೆ ಸಮ್ಮತಿ ನೀಡಲು ಒತ್ತಾಯಪಡಿಸಿಲ್ಲ ಅವರು ಸ್ವತಂತ್ರವಾಗಿ ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸಿದ ಬಗ್ಗೆ ನಾನು ಖಾತ್ರಿ ಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಸಮ್ಮತಿ ಹೇಳಿಕೆ ನಮೂನೆ ಪ್ರತಿಯನ್ನು ಭಾಗವಹಿಸುವವರಿಗೆ ಕೊಟ್ಟಿದೆ.

ಸಂಶೋಧನಾಕಾರರ ಹೆಸರು : _____

ಸಹಿ : _____

ದಿನಾಂಕ : _____
ದಿವಸ/ತಿಂಗಳು/ವರ್ಷ

के. एल. ई सोसायटीचे जे. एन. वैद्यकिय कॉलेज , नेहरु नगर, बेळगावी

भाघिदारांची माहिती पत्र

हे भाघिदारांची माहिती पत्र अशा महिलांसाठि आहे, ज्यांनी प्रसुतीसाठी प्रवेश घेतला आहे. आणि त्यांना आम्ही रक्तवाहिनींच्या वाढीसाठी आवशक घटकांची वर होणा–या संशोधनामध्ये सहभागी होण्यासाठी आमंत्रीत करत आहोत.

प्रकल्पाचा मथळा : "इम्युनोहीस्टोरीकेमीकल मुल्यमापन रक्तवाहिनींच्या वाढीसाठी आवशक घटकांची अभ्यास व त्याचां वार व बाळचां वाढीवर होणारा परीणाम अभ्यासाणे"

संशोधकाचे नावं :मिसेस वनिशा
संस्थेचे नावं : शरीररचना शास्त्र विभाग
के. एल .ई चे जे एन. मेडीकल कॉलेज बेळगावी

माहितीपुर्व संमतीपत्राचे दोन भाग आहेत.

- माहीतीपत्र तुम्हाला या संशोधनाबद्द्लची माहिती देते
- संमतीपत्र (तुमची सहमती असेल तर हस्ताक्षर करण्यासाठी)
  तुम्हाला या माहीतीपूर्व संमतीपत्राची एक प्रत देण्यात येईल.

माहिती पत्र :

ओळख ; मी वनिता जे एन एम सी बेळगाविच्या शरिर रचना शास्त्र विभागामध्ये पी. एच डी ची पूर्णवेळ विद्यार्थिनी आहे. आम्ही वार मधिल व स्कूलर एन्डोथीलायन ग्रोथ फ़ॅक्टर विषयी संशोधन करीत आहोत. वार हे मातेला आणि अर्भकाला जोडणारे अवयव आहे. वार विकासामध्ये होणारा बिघाड हा अर्भकाच्या विकासामध्ये बदल घडवू शकतो. हा अभ्यास यासाठी केला जात आहे. की, ज्याद्वारे रक्तवाहिनींच्या वाढीसाठी आवशक घटकांची चे वार मधेल अभिव्यक्ती जे रक्ताची जडणघडणीसाठी मदत करते ज्यामूळे अर्भकाच्या वाढीसाठी मदत होते.

येथे असे कांही शब्द असतील जे तुम्हाला समजू शकणार नाहीत. तेव्हा तुम्हाला मी माहिती सांगत असताना कृपया थांबवा आणि , मी तुम्हाला समजवून सांगू शकेन जर तुम्हाला या माहितीनंतर प्रश्न असतील तर ते तुम्ही मला विचारु शकता.

संशोधनचा उद्देश:

वाढीचा घटाकांचा वारचा विकासामध्ये महत्वाची भूमिका आहे आणि त्यांचा तील बिघाड हे प्लॅसेंटा पॅथालाजीसशी संबंधीत असते. हे रक्तवाहीण्या विकासामध्ये मदत करते. त्यामुळे मातेकडून अर्भकाला होणा–या पौष्टीक पदार्थाच्या प्रमाणात वाढ करते जे, की अर्भक विकसित होण्यासाठी आवश्यक आहे. हा अभ्यास त्याच्या कार्यावर आणि पातळीवर लक्ष केंद्रित करतो. सामान्य शरीर विज्ञान शास्त्र समजण्यास मदत होते. आणि गर्भावस्थेमधील गुंतागुंतीची कारणे शोधण्यास मदत होते.

ऐच्छीक सहभाग :

तुमचा या संशोधनामधिल सहभाग हा पूर्णपणे ऐच्छीक अजून यात भाग घ्यावा कि न घ्यावा हा तुम्चा निर्णय आहे याचा हॉस्पिटल मधील सेवेवर कोणताही परिणाम होणार नाही आणि त्यामध्ये बदळही होणार नाही.

कार्यपध्दती :

या संशोधनात आम्ही बाळंतपणानंतर वार जमा करून त्याची चाचणी करतो. यात वार चे वजन, आकारमान, व्यास यांची नोद करतो आणी छोटासा भाग वापरून त्यातील रक्तवाहिनीशी संबंधीत घटकांचा अभ्यास करतो. त्यामुळे वार त्याची व बाळाची वाढ, वार ची कार्य पदधती या गोष्टी समजण्यास मदत होते. त्याचबरोबर अभ्यासामध्ये तुम्हाला तुमची सध्याचि गरोदर अवस्था, पुर्विच्या गरोदर अवस्था आणि आरोग्य उपचार घेतल्या संबन्धी लिहीलेल्या माहितिवर प्रश्न विचारले जातील त्यावर तुम्ही उतर देण्याचे आहे. आम्ही तुमच्या अर्भकाचाही मोजमाप घेऊ. जसा की वजन, उंची आणि परीघ.

कार्यपध्दतीचा अवधी :

जर तुम्हि सहभागी होण्यास संमति दिली तर संपुर्ण कार्यपध्दतीला १५ मिनीटे लागतील.

धोके : या अभ्यासामध्ये कोणताही धोका नाही . कारण हा अभ्यास फ़क्त प्रश्नांवर आधारित आहे, ज्यांची तुम्ही उतरे द्यावयाची आहेत. यामध्ये कोण्तीही शारिरीक तपसली केलि जाणार नाही.

फायदे :

संशोधनामधील प्रश्नांची उकल करण्यासाठी तुमचा सहभाग आम्हाला उपयोगी ठरु शकतो. जरी या अभ्यासामुळे समाजाला या टप्प्यावर कोणत्याही फायद संभवीत नसला तरी भावी पिढयांना याचा फायदा होणार आहे.

नुकसान भरपाई :

या अभ्यासामध्ये सहभागी होण्यासाठी तुम्हाला कोणताही मोबदला मिळणार नाही. या अभ्यासामध्ये भाग घेण्यासाठी तुम्हाला कोणताही अतिरिक्त खर्च सहन करावा लागणार नाही. या अभ्यासाचा खर्च पूर्णपणे संशोधकांचा राहील.

गोपनियता:

या संशोधन प्रकल्पातून जमा झालेली माहिती गुप्त ठेवली जाईल. तुमच्याकडून घेललेली माहीती बाजूला साठवून ठेवली जाईल. आणि फक्त संशोधकालाचा तिची पाहणी करता येईल. तुमच्या बद्दलच्या कोणत्याही माहितीवर तुमचे नांव नसेल तर फक्त एक क्रमांक असेल. फक्त संशोधकालाच तुमचा क्रमांक माहिती असेल आणि आम्ही ती माहिती गौप्य ठेवू ती कुणाकडे ही उघड केली जाणार नाही अथवा कुनालाही दिली जाणार नाही.

निकालांची हाताळणी :

या अभ्यासामधून आम्हाला जी माहिती मिळले ती जनतेला देण्याअगोदर संशोधन मंडळासमोर मांडली जाईल. गोपनीय माहिती उघड केली जाणार नाही संशोधन कमीटीसमोर मांडल्यानंतर आम्ही तो निकाल प्रकाशित करू त्यामुळे यामध्ये रस असलेल्या लोकांना आमच्या संशोधनामधून आवश्यक माहिती मिळविता येईल

माघार धेण्याचा:

तुम्हाला या संशोधनामध्ये भाग घेण्याची इच्छा नसेल तर तसे करण्यास तुम्ही मोकळे आहात. या संशोधनानमध्ये भाग घेण्याचे कोणत्याही वेळेस तुम्ही नाकारु शकता तो.

कोणाशी संपर्क साधाल :

जर तुम्हाला कोणतेही प्रश्न असले तर तुम्ही ते आता वा नंतर विचारु शकता. अगदी हा अभ्यास चालू झाल्यानंतर देखील. जर तुम्हाला नंतर काही प्रश्न विचारायचे असल्यास तुम्ही पुढील कोणाशीही संपर्क करू शकता.

1. मिसेस वनिथा, मोबईल नं 8095600731

2. डॉ दक्षा दिक्षित, शरीर रचना शास्त्र विभाग,
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3. डॉ. अनिल होगडे, चेअरमन , एथीकल कमिटी
   के. एल. ई युनिव्हर्सिटी , बेळगावी, मोबाईल नं. 0831-2473777
   एक्स्टेंशन 4064/4095

कायदेशिर हक्क

या संमति पत्रावर हस्ताक्षर केल्यामुळे तुमच्या कोणत्याही कायदेशिर हक्कला बाधा पोहचत नाही.

सम्मती पत्र

मी सदर माहीती वाचलेली आहे, / मला वाचून सांगण्यात आली, आहे, मला सदर विषया बाबत प्रश्न करणेस संधी असून मला विचारलेल्या प्रश्नांना उत्तर दिले आहे. मी, स्व–खुषीने या संशोधन अभ्यास क्रमात भाग घेणेस सम्मती दिली आहे.

भाग घेणाऱ्यांचे नाव: _____

भाग घेणाऱ्यांची सही : _____

दिनांक : _____
दिवस/महीना/ वर्ष

अशिक्षीत असल्यास :

भाग घेणाऱ्यांना सदर विषया बाबत सर्व माहीती सविस्तर वाचून सांगीतली असून व त्यांनी त्या बद्दल संमती दिली असुन व सदर यांना या विषया बाबत प्रश्न करणेस सोय असुन भाग घेणाऱ्यानी स्वतंत्रपणे आपले समंती दिल्या बद्दल मी साक्षी आहे.

साक्षीदारांचे नाव : _____ व डाव्या हाताचा ठस्सा

साक्षीदारांचे सही _____

दिनांक : _____
दिवस/महीना/ वर्ष

संशोधकाचा जवाब :

मी, सूक्ष्मपणे या अभ्यास क्रमात भाग घेणाऱ्यांची माहिती विषय वाचून घेतली आहे. व त्यात भाग घेणाऱ्यानी सर्व विषय समजून घेतल्या बद्दल मला खात्री आहे. भाग घेणाऱ्याना सदर विषया बाबत अवकाश असल्या बाबत मी खात्री करुन घेतले आहे. त्यानी विचारलेले सर्व प्रश्नाना योग्य उत्तर दिलो आहे.

व सदर यांना समंती देणेस कोणताही दबाव केला नाही. त्यानी स्व–खुषीने स्वयम् इच्छापूर्वक सम्मती दिल्या बाबत खात्री करुन घेतलो आहे.

सम्मती जवाब नमुना प्रत भाग घेणाऱ्याना दिली आहे.

संशोधनकारांचे नाव : ___ _____

संशोधनकारांची सही : __ _____

दिनांक : _____
दिवस/महीना/ वर्ष

ANNEXURE – III – ETHICAL CLEARANCE LETTER

KLE UNIVERSITY

(Formerly known as KLE Academy of Higher Education & Research, Belagavi)
[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956 vide Government of India Notification No.F.9-19/2000-U.3(A)]
'Accredited **'A' Grade** by NAAC (2nd Cycle) Placed in **Category 'A'** by MHRD (GoI)

Office of the **Director, Academic Affairs**

JNMC Campus, Nehru Nagar, Belavi-590 010, Karnataka State, India

☎: 0831-2444444/2493779 **FAX:** 0831-2493777 **Web:** http://www.kleuniversity.edu.in **E-mail**:diracademic@kleuniversity.edu.in

Ref.No.KLEU/EC/17-18/D- 30 26th April 2017

To,
Ms. Vanitha
Full Time Research Scholar,
2016-17 batch, Faculty of Medicine
J. N. Medical College, **Belagavi**

Dear Research Scholar,

Sub:- Regarding Ethical Clearance.

The KLE University **Ethics Committee on Human Subjects** for Ph. D Research Project met on **22nd March 2017** to consider your application for approval of the research project **" Immunohistochemical evaluation of vascular endothelial growth factor – A (VEGF-A) in human placenta and its role in growth of placenta and fetus.."**

As there are no ethical issues involved in your proposed research project, the committee has provided approval for this research project.

You are requested to report to Ethical Committee in case of the following:

1. Any deviation from or change of the protocol.
2. All serious adverse events.
3. Any changes in study documents.

28(4)17

(Dr. Anita Dalal)
Member Secretary,
Ph.D. Ethical Committee(Human),
K.L.E. University,
Belagavi.

(Dr. Anil Hogade)
Chairman
Ph.D. Ethical Committee(Human),
K.L.E. University,
Belagavi.

(On A.P. Hogade)

CC to: - The Director Academic Affairs, KLE University, Belagavi.
- The Director Research Foundation, KLE University, Belagavi.
- The Registrar, KLE University, Belagavi

ANNEXURE – IV – PUBLICATIONS

International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Expression of Vascular Endothelial Growth Factor-A 165 (VEGF-A) in Term Normal Placenta: Immunohistochemical Study

Vanitha[1], Dr. Daksha Dixit[2], Dr. Adarsh Sanikop[3], Dr. R. D.Virupaxi[4]

[1]PhD Scholar, [2]Professor, [4]Professor& Head, Department of Anatomy,
[3]Assistant Professor, Department of Pathology,
Jawaharlal Nehru Medical College, KLE Academy of Higher Education & Research (KAHER), Belagavi, Karnataka, India.

Corresponding Author: Vanitha

ABSTRACT

Introduction: Vascular Endothelial Growth Factor (VEGF) is a potent angiogenic growth factor and acts as a key regulator of vascular development, implicated in the development of both pathological and physiological angiogenesis. In the placenta, it has been identified recently and considered as one of the important angiogenic growth factors in the development of the embryo. We have taken up this study to see the expression of VEGF-A 165 in normal placenta by immunohistochemistry.
Materials and methodology: One hundred central sections of placentae slides were deparaffinized followed by peroxidase block and protein block. After this, the slides were incubated in VEGF mouse monoclonal antibody for 1 hr followed by incubation in Horseradish peroxidase (HRP) for 30 mins and Diaminobenzidine (DAB). Stained sections were observed for VEGF expression in the villi of placental tissues.
Results: Expression of VEGF was observed in syncytiotrophoblast, endothelium of blood vessels and hofbauer cells of villi of the placenta.
Conclusion: In this study, we studied the expression of VEGF in normal term placenta of 37-41 weeks of gestation. The VEGF expression on syncytiotrophoblast and hofbauercells can act on paracrine mode on vessels to modulate angiogenesis, to maintain endothelium and permeability and through autocrine mode to influence trophoblast function during pregnancy.

Key words: Placenta, terminal villi, VEGF.

INTRODUCTION

Vascular endothelial growth factor is an angiogenic growth factor (VEGF), Disulphide-linked homodimeric glyco-protein which was first identified as a vascular permeability factor. Later, in 1989, Napoleone Ferrara and his colleagues at Genentech, isolated and cloned the vascular endothelial growth factor from bovine pituitary follicular cells. It is a potent angiogenic growth factor and acts as a key regulator of vascular development, [1] implicated in the development of both pathological and physiological angiogenesis. [2] In the placenta, it has been identified recently and considered as one of the important angiogenic growth factors in the development of the embryo. There are different types of VEGF which have been identified, like VEGF-A, PlGF, VEGF-B,VEGF-C VEGF-D andVEGF-E, [3] but only VEGF-A,PLGF, VEGF-B and VEGF-C have been identified in placenta. [4] VEGF-A is also called as VEGF. [5] There

are different isoforms of VEGF-A like VEGF-A121, VEGF-A145, VEGF-A165, VEGF-A183, VEGF-A189 and VEGF-A 206 having 121, 145, 165, 183, 189 and 206 amino acids. [6] VEGF binds 2 tyrosine kinase receptors, Vascular Endothelial Growth Factor Receptor-1 (VEGFR-1 or Flt-1) [7] and Vascular Endothelial Growth Factor Receptor-2 (VEGFR -2 or KDR) [8] which are present in endothelial cells. In addition, it also binds Neuropilin -1(NRP-1) and Neuropilin-2 (NRP-2). [6] An in-vitro study has shown the role of VEGF in spiral artery remodeling, trophoblast invasion during implantation and also in the reduction of apoptosis, thereby helps in angiogenesisandvasculogenesis. [9] The growth factors which stimulate and maintain vascular growth and function have developmental and clinical significance. There are reports on the expression of VEGF in placenta, but studies showed discrepancies in the expression of VEGF, so we have taken up this study to see the expression of VEGF-A 165 in normal placenta by immunohistochemistry.

MATERIALS AND METHODS

One hundred normal placentae of 37-41 weeks of gestational age were collected, after taking an informed consent. A section of placenta was taken from the center and fixed in neutral buffered formalin for 24 hrs. After fixing the tissue, the tissue was processed for the block preparation. Then it was sectioned into 3µm and transferred ontopositively coated slides. For immunostaining, the slide was incubated overnight at 37°C and in the morning it was kept on a hot plate for fixing at 63°C for 1hr. Then the slide was processed for deparaffinization followed by peroxidase block and protein block. After this, the slide was incubatedin VEGF mouse monoclonal antibody (Diagnostic Biosystems USA Pleasanton, catalog # PDM165-RUO) for 1hr followed by incubation in HRP (Biocare Medical, USA) for 30 mins and DAB (Biocare Medical, USA). Positive (Kidney tissue) and negative control slides (Reagent control) were run together with the sample tissue. Stained section was observed for VEGF expression in the villi of placental tissues.

RESULTS

The VEGF-A staining was observed in syncytiotrophoblast, endothelium of blood vessels and hofbauer cells of villi of placenta (Figure 1 & 2). There were no differences in the expression of VEGF in 37-41 weeks of gestation.

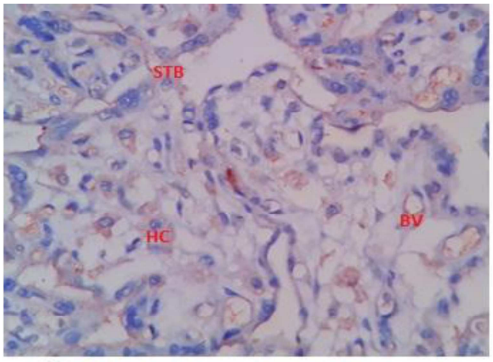

Figure 1: VEGF expression in blood vessels (BV), hofbauer cells (HC) and syntiotrophoblast (STB) layer of villi of placenta

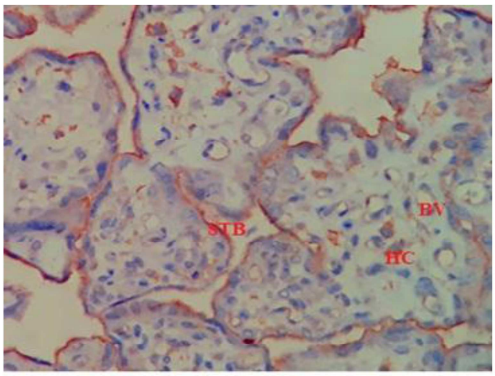

Figure 2: VEGF expression in blood vessels (BV), hofbauer cells (HC), syntiotrophoblat (STB) layer of villi of placenta

DISCUSSION

Development of vessels in the placenta, as well as in maternal endometrial tissue is required to maintain the pregnancy and fetal growth, which are regulated by the angiogenic growth factors. Alterations in the

expression of these growth factors can affect the development of the fetus. VEGF is considered to be one of the dominant angiogenic growth factors in the development of vessels in the placenta. In this immunohistochemical study, we tried to find expression of VEGF-A in the normal placenta of 37-41 weeks of gestation. The VEGF expression was found in the syncytiotrophoblast layer, endothelial cells of blood vessels and hofbauer cells of villi of placenta. Earlier studies on early and term normal placenta showed,VEGF in hofbauer cells and decidual cells, glandular epithelium and extravillous trophoblast (EVT) in the uterus of the first trimester. In term, VEGF was found in syncytiotrophoblast, EVT and in the extracellular material. Expression of VEGF by hofbauer cells can act on endothelial cells for the capillary formation and vessel permeability throughout gestation and Flt on hofbauer cells may act on autocrine manner to control their function. The expression on EVT, decidual cells and glandular epithelium suggests its role in EVT migration and diffrentiation. [10] In another study, theexpression was observed insyncytiotrophoblast, cytotrophoblast, endothelial cells, EVT, hofbauer cellsand diffused staining within the villous in term placenta. More staining was observed in cytotrophoblast than that in syncytiotrophoblast. [11] Vuorela et al observed protein expression in villous blood vessels and less or no staining in the villous stroma and mRNA expression in mesenchymal cells of chorionic plate. Hence the cells of chorionic plate secrete VEGF, that diffuses through the villi and acts on the endothelium of vessels of villi in paracrine mode. [12] In one of in-situ hybridization studies of the placenta of first and third trimester revealed, VEGF mRNA expression on syncytiotrophoblast, cytotrophoblast and hofbauer cells of mesoderm, may be involved in the angiogenesis of fetal blood vessels during placental growth. Expression was also observed in decidua of the first-trimester uterus, in glandularepithelium anduteroplacental junction adjacent to Nitabuch's stria. In term placenta, the expression of VEGF mRNA was seen in syncytiotrophoblast cells and inhofbauer cells. This study concluded that macrophages are the primary source of VEGF. [13] Our study results are consistent with the mRNA expression of VEGF in syntiotrophoblast and hofbeaur cells, but not with the endothelial cells. The endothelial expression may be due to the presence of receptors of VEGF by which VEGF acts on it. [10] One of the in-vitro studiesshowed expression of VEGF in cytotrophoblast and syntiotrophoblast by ribonuclease protection assay (RPA). However, the expression was lower compared to PLGF. Under hypoxic condition, the expressionof VEGF was increased in cultured cells. [14] VEGF is important inthe development of blood vessels in the placenta and placental tissue.There was a positive correlation between the expression of VEGF and blood vessels /unit area of tissue in the pig. Increased blood vessels at the placental endometrial interface results in an increased nutrient transfer between mother and fetus. [15] One of the study showed increased expression of VEGF in blood vessels of villi of placenta under pathological conditions. [16] In another study, VEGF mRNA was localized to cytotrophoblast, syncytiotrophoblast, stromal cells and in perivascular cells of first, second and third trimester placentae. But in preeclampsia, VEGF was found mainly in syncytiotrophoblast and fibrous stroma. An increased level of VEGF was observed in preeclampsia. In this study, they found a difference in the expression of VEGF in normal and preeclampsia and their results were consistent with mRNA expression. [17] Isolated macrophages from villi have shown expression of VEGF, which may have dual immune and morphogenetic role on the placenta. [18] In this study, we found expression of VEGF in syncytiotrophoblast, blood vessels of the villi and hofbauercells of the villous stroma of villi of placenta.

The VEGF expression on syncytiotrophoblast and hofbauercells can act on paracrine mode on vessels to modulate angiogenesis, to maintain endothelium and permeability and through autocrine mode to influence trophoblast function during pregnancy. [13,14]

CONCLUSION

In this study, we observed the expression of VEGF in syncytiotrophoblast, blood vessels of the villi and hofbauer cells of villi of normal term placenta of 37-41 weeks of gestation. This will help to understand its expression and function in normal pregnancy.

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How to cite this article: Vanitha, Dixit D, Sanikop A et.al. Expression of Vascular Endothelial Growth factor-A 165 (VEGF-A) in term normal Placenta: Immunohistochemical study. Int J Health Sci Res. 2018; 8(8):110-114.

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Vanitha et al., IJSRR 2019, 8(1), 4865-4874

Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Immunohistochemical Study of Differential Expression of VEGF - A in the Normal Placenta and its Association with Fetal and Placental Parameters in Term Pregnancy

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ABSTRACT

Vascular Endothelial Growth Factor (VEGF) is a key molecule in the development of vessels in the placenta. In early pregnancy, it helps in implantation, development of vessels in the villi of placenta and formation of terminal villi i.e. in non-branching angiogenesis. These are necessary for placental development and fetal growth. Studies have reported altered expression of VEGF in complicated pregnancy. To understand pathology, there is a need to understand its expression level in the normal placenta and its association with the fetal growth parameters.

Placentae of 32-41 weeks of pregnancy were collected. Sections of placentae were stained with the antibody VEGF-A165. The fetal and placental growth parameters were also recorded. The expression of VEGF was quantified by using Image analyses software.

The expression level of VEGF was more in Hofbauer cells (HC). The VEGF level did not change with the gestation from 32-40 weeks. But in 41 weeks, there was an increase in the level of VEGF. The expression of VEGF was more in the male fetus. We also noticed the expression of VEGF did not change with fetal and placental growth parameters.

The expression of VEGF is stabilized at the end of pregnancy, but in late term, there was an increase in the level due to decreased oxygen level in the placenta. The VEGF level at the end of pregnancy may not be useful in the prediction of growth parameters.

KEYWORDS: Angiogenesis, Fetal growth, Placenta, Vascular endothelial growth factor, Vasculogenesis.

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INTRODUCTION

Vascular Endothelial Growth Factor (VEGF) is a potent angiogenic growth factor and vascular permeability factor, prominently expressed in the placenta and helps in the development of vessels in the placenta[1]. VEGF helps in implantation, spiral artery remodeling[2] and also in Nitric Oxide (NO) secretion which is a vasodilator, helps in vasodilation of vessels and increases the cardiac output and plasma volume of the mother thereby helps in fetal growth[3,4]. Abnormal pregnancy outcomes are tagged with the vasculogenesis and angiogenesis in the placenta[5,6,7,8]. Imbalance in the angiogenic factors will affect the vascular development which will reflect on placental development and fetal growth[9]. The normal expression of VEGF is important in the healthy growth of the embryo and in pregnancy outcome. Altered expression of VEGF was reported in complicated pregnancies[10,11,12,13,14,15]. But the results are varied. Therefore to understand the pathology behind complications, it is important to study the VEGF normal expression and its association with the fetal growth parameters. Studies have reported maternal serum expression of VEGF in early, and in mid-gestation, and its association with fetal and placental growth parameters[16,17]. There are no data on the expression of VEGF in different stages of term pregnancies. Therefore, this study has been undertaken to study its expression in term pregnancies and also to check changes in the expression of VEGF with placental and fetal growth parameters.

METHODOLOGY

One hundred and seventy three placentae were collected from the Department of Obstetrics and Gynecology, Dr. Prabhakar Kore Charitable Hospital, Belagavi, after taking informed consents. Institutional ethical committee permission was obtained for the study. Placentae of 32-41 weeks of gestation were collected for the study. Mothers with complications like hypertensive disorders of pregnancy, gestational diabetes and all other complications were excluded from the study.

Recording maternal history

Maternal history was recorded on a predesigned proforma.

Recording placental and fetal parameters

Placental parameters were measured after trimming cord and membranes. The weight of the placenta was measured on weighing balance, volume by water displacement method and surface area was measured by taking smallest and largest length by using a measuring tape, then the surface area was calculated by the formula; Surface area= π x dl x ds /4.

The fetal weight was measured on weighing machine, length by infantometer and head circumference by using a measuring tape.

Immunohistochemical procedure

A central section of placenta was fixed in neutral buffered formalin for 48 hrs and then processed for block preparation. The tissue blocks were sectioned at 5µm and the slides were processed for immunohistochemical staining. Here we used mouse monoclonal antibody VEGF-A165 from Diagnostic Biosystems, USA Pleasanton and a secondary kit from Biocare Medical, USA. The protocol was followed according to company instructions.

Observation of slides and interpretation

The VEGF is a cytoplasmic and extracellular protein. Staining was observed in the cells of the villi of the placenta. The VEGF staining was found in syncytiotrophoblast (STB), Hofbauer cells (HC) and endothelium of blood vessels (BV) (Published data- qualitative data)[18]. Depending on the expression, the intensity was measured from these cells in Image analyses software.

Statistical analyses

Statistical analyses were performed in SPSS software version 23. First, we observed for distribution of variable by applying the chi-square test. The mean and SD were taken for continuous variable. Then we applied z-test to see the differences between the groups, $p<0.05$ was considered to be significant.

RESULTS

Maternal and fetal demographic data

Mean age of the mother was 24.36±3.37, 47.98% mothers were primipara and 52.02% of mothers were multipara. In this study, 58.96% were delivered normally and 41.04% had a cesarean delivery.

Mean gestational age was 38.35±1.74, mean birth weight was 2784.45±347.11, mean fetal length was 48.59±3.14 and mean head circumference was 33.85 ± 1.87, 48.55% were male babies and 51.45% were female babies.

Mean placental weight was 445.03±87.03, mean placental volume was 440.04±87.05 and mean placental surface area was 246.89±49.19.

VEGF expression and its relation with growth parameters

The total intensity of VEGF in STB cells was 113.25±9.11, HC cells was 114.66±11.52 and BV cells was 115.89±7.90. The cellular expression of VEGF was more in HC cells which was statistically significant at $p<0.001$.

Expression of VEGF with maternal data

Expression of VEGF did not change with maternal age and parity.

Gestation-wise changes in VEGF expression

The VEGF expression did not change from 32-40 weeks of gestation, their differences were not statistically significant ($p>0.05$). In 41+ weeks of gestation, the expression of VEGF was significantly increased which was statistically significant ($p<0.001$) **(Table No. 1).**

Table No. 1: Shows expression of VEGF from 32-41 weeks of gestation

Gestational age group		Intensity of syncytiotrophoblast (STB)	Intensity of blood vessels (BV)	Intensity of Hofbauer cells (HC)	Total VEGF intensity
<37	N	25	25	25	25
	Mean	109.56	116.02	116.65	114.07
	SD	8.23	10.07	6.54	6.21
37-38	N	59	59	59	59
	Mean	113.37	113.93	121.78	116.36
	SD	11.46	12.42	8.74	9.11
39-40	N	77	77	77	77
	Mean	113.41	115.07	117.32	115.26
	SD	7.41	12.09	7.59	7.65
41+	N	12	12	12	12
	Mean	119.33***	112.86***	132 ***	121.40**
	SD	2.6	4.38	4.73	2.12

(*p<0.001, **p<0.01, *p<0.05, ns= Not significant)**

VEGF expression by fetal parameters

We compared VEGF expression with the fetal parameters like sex **(Table No. 2)**, birth weight **(Table No. 3)**, length of the fetus **(Table No. 4)** and head circumference **(Table No. 5)**. Placenta of male babies showed a significant increase in VEGF in BV ($p<0.05$) and HC cells. But, the increased expression of VEGF in HC cells was not statistically significant. We did not see changes in the expression of VEGF by fetal growth parameters. The differences were not statistically significant ($p>0.05$).

Table No. 2: Expression of VEGF by sex of fetus

Sex of fetus		Intensity of syncytiotrophoblast (STB)	Intensity of blood vessels (BV)	Intensity of Hofbauer cells (HC)	Total VEGF intensity
Male	N	84	84	84	84
	Mean	113.30	116.49*	120.23	116.67
	SD	10.02	12.34	8.35	7.97
Female	N	89	89	89	89
	Mean	113.20	112.94	119.33	115.15
	SD	8.22	10.46	8.91	7.80

(*p<0.001, **p<0.01, *p<0.05, ns= Not significant)**

Table No. 3: Expression of VEGF by birth weight

Birth weight group[ns]		Intensity of syncytiotrophoblast (STB)	Intensity of blood vessels (BV)	Intensity of Hofbauer cells (HC)	Total VEGF intensity
2000-2499	N	36	36	36	36
	Mean	113.84	116.75	119.67	116.75
	SD	10.41	7.93	8.22	6.79
2500-2999	N	90	90	90	90
	Mean	112.03	114.76	119.70	115.49
	SD	8.25	12.05	8.47	7.49
3000+	N	47	47	47	47
	Mean	115.15	112.89	119.96	116.00
	SD	9.19	12.68	8.67	9.21

(*p<0.001, **p<0.01, *p<0.05, ns= Not significant)**

Table No. 4: Expression of VEGF by length of fetus

Fetal length group [ns]		Intensity of syncytiotrophoblast (STB)	Intensity of blood vessels (BV)	Intensity of Hofbauer cells(HC)	Total VEGF intensity
<45	N	17	17	17	17
	Mean	113.90	116.14	121.34	117.13
	SD	11.07	10.80	8.49	7.92
45-56	N	156	156	156	156
	Mean	113.18	114.50	119.59	115.76
	SD	8.70	11.59	8.62	7.85

(*p<0.001, **p<0.01, *p<0.05, ns= Not significant)**

Table No. 5: Expression of VEGF by head circumference of fetus

Head circumference group [ns]		Intensity of syncytiotrophoblast (STB)	Intensity of blood vessels (BV)	Intensity of Hofbauer cells (HC)	Total VEGF intensity
<32	N	35	35	35	35
	Mean	112.85	116.08	119.09	116.01
	SD	9.61	9.89	6.73	6.68
33-35	N	117	117	117	117
	Mean	112.80	114.33	119.62	115.58
	SD	8.53	12.07	8.97	8.10
36+	N	21	21	21	21
	Mean	116.44	114.14	121.69	117.42
	SD	11.08	11.20	9.63	8.76

(*p<0.001, **p<0.01, *p<0.05, ns= Not significant)**

VEGF expression by Placental parameters

We compared expression of VEGF by placental parameters. The expression of VEGF did not change with the placental weight **(Table No. 6)**, placental volume **(Table No.7)** and surface area **(Table No. 8)**.

Table No.6: Expression of VEGF by placental weight

Placental weight group [ns]		Intensity of syncytiotrophoblast (STB)	Intensity of blood vessels (BV)	Intensity of Hofbauer cells (HC)	Total VEGF intensity
<400	N	50	50	50	50
	Mean	113.25	115.77	118.42	115.81
	SD	9.49	12.67	9.86	8.42
400-600	N	114	114	114	114
	Mean	113.20	114.61	120.33	116.04
	SD	8.98	10.70	7.92	7.51
600+	N	9	9	9	9
	Mean	113.92	109.27	120.10	114.43
	SD	9.46	13.43	9.57	9.78

(*p<0.001, **p<0.01, *p<0.05, ns= Not significant)**

Table No. 7: Expression of VEGF by volume of placenta

Placental volume group [ns]		Intensity of syncytiotrophoblast (STB)	Intensity of blood vessels (BV)	Intensity of Hofbauer cells (HC)	Total VEGF intensity
<400	N	57	57	57	57
	Mean	113.90	117.05	118.28	116.41
	SD	9.80	12.13	8.71	7.93
400-600	N	105	105	105	105
	Mean	112.58	113.57	120.23	115.46
	SD	8.64	10.82	8.26	7.65
600+	N	10	10	10	10
	Mean	115.34	111.43	121.45	116.07
	SD	9.73	12.91	9.97	9.55

(*p<0.001, **p<0.01, *p<0.05, ns= Not significant)**

Table No. 8: Expression of VEGF by placental surface area

Placental surface area [ns]		Intensity of syncytiotrophoblast (STB)	Intensity of blood vessels (BV)	Intensity of Hofbauer cells (HC)	Total VEGF intensity
<200	N	26	26	26	26
	Mean	112.36	114.84	116.73	114.31
	SD	9.88	14.59	8.79	8.88
200-300	N	121	121	121	121
	Mean	113.37	114.93	120.42	116.24
	SD	9.20	11.27	8.47	7.86
300+	N	25	25	25	25
	Mean	114.12	113.35	120.92	116.13
	SD	7.56	9.37	8.53	7.07

(*p<0.001, **p<0.01, *p<0.05, ns= Not significant)**

DISCUSSION

In the present study, we observed the expression of VEGF in the placenta of 32-41 weeks of gestation and compared their expression with the fetal and placental parameters. Maternal age and parity did not affect the VEGF expression in this study. The VEGF expression did not change from 32-40 weeks of gestation, the differences between them were not statistically significant, and in 41+

weeks of gestation (late term), we observed a significant increase in VEGF expression in the three cells, which was statistically significant ($p<0.001$). We found increase in VEGF expression in male fetus in BV and HC cells. When we compared the expression level of VEGF with fetal and placental growth parameters, we did not see changes in the expression of VEGF with the growth parameters. One of the study showed increased pro-inflammatory and angiogenic factors (Placental growth factor (PlGF),VEGF) in women carrying male fetus because male baby carrying women shows more pro-inflammatory and pro-angiogenic immune response[19], may be the reason of increased VEGF expression in this study. Lygnos et al studied maternal serum level of VEGF in first, second, third trimester and at the day of delivery observed that the expression of VEGF was increased in the first trimester, declined thereafter[16]. Sundrani et al also reported similar findings, the maternal serum VEGF level was increased from 16-20 to 26-30 weeks of gestation thereafter expression level was reduced[17]. These studies explain that the initial rise in the VEGF is due to decreased oxygen in developing placenta, the VEGF is a hypoxia-inducible factor and the decreased oxygen stimulates VEGF secretion. As the blood vessel formation increases, the oxygen level becomes stable through vasodilation of blood vessels by NO so the VEGF level decreased at the end of gestation[16]. In our study also, we did not observe any changes in the level of VEGF from 32-40 weeks of gestation. At the end of pregnancy, blood vessel formation is stabilized and sufficient oxygen supply may be the reason we did not see changes in the level of VEGF. But in 41+ weeks of gestation (late term), VEGF is significantly increased than in the 32-40 weeks of gestation, this may be due to the fact that after 40 weeks of gestation, calcium starts depositing on blood vessels and protein gets deposited on the placenta, which limits the blood supply through the placenta leading to placental insufficiency which may be the reason of increase of VEGF in late-term pregnancies[20]. In the present study we also compared the expression of VEGF with birth weight, fetal length and head circumference and also with the placenta weight, placental volume and surface area. We did not see changes in expression of VEGF with these growth parameters. In one of the study, a positive correlation was found between early expression of VEGF at 16-20 weeks of gestation with birth weight and expression of VEGF at 26-30 weeks of gestation with the fetal length. The level of VEGF at the time of delivery did not show association with the birth weight, length, head circumference and chest circumference in this study. This study also concludes that early expression of VEGF at 16-20 weeks can be used to predict the birth weight[17]. In one of the study, an association was found between maternal serum VEGF level at mid-gestation (12-27 weeks) with the placental weight[9]. Wheeler et al found a positive correlation between VEGF concentrations at 16-20 weeks with placental volume at 16-20 weeks ultrasonography measurements. The VEGF level at 16-20 weeks was positively correlated with the birth weight and placental weight at delivery. The VEGF level was elevated up to

20 weeks of gestation[21]. In our study, we collected placenta of 32-41 weeks of gestation, where its expression did not change and at the end of pregnancy VEGF expression is reduced according to above literature, this may be the reason we did not see the relation with the growth parameters and VEGF expression. This was an immunohistochemical study, further similar studies may be required for the validation of our study findings.

CONCLUSION

The expression of VEGF is stabilized at the end of pregnancy, but in late term, there was an increase in the level due to decreased oxygen level in the placenta. The VEGF level at the end of pregnancy may not be useful in prediction of growth parameters.

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Biomedicine: 2019; 39(1): 110-112

Study on Blood Vessels of Terminal Villi of Normal Term Placenta

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(Received: May 2018 Revised: Aug 2018 Accepted: Dec 2018)

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ABSTRACT

Introduction and Aim: Terminal villi form the functional units of the placenta. The terminal villi consist of a layer of cytotrophoblast and syncytiotrophoblast with a core of mesoderm with blood vessels. These villi form the placental barrier and transfer nutrients from mother to fetus and waste products from fetus to mother. Successful pregnancy depends upon the formation of blood vessels in the placenta to supply the increasing demand for nutrition for developing the embryo. Inadequate vessel formation can result in impaired placental growth, malfunction, hypoxia, and fetal malnutrition that may result in fetal death. This study has been taken up to study the normal vasculature of the terminal villi of term placenta.

Materials and Methods: One hundred and fourteen normal placentae of 37-41 weeks of gestational age were collected and stained with Hematoxylin and Eosin.

Results: The mean of blood vessels/terminal villi was 6.88 with a standard deviation of 1.39. The number of vessels/terminal villi was reduced from 37 - 41 weeks of gestation.

Conclusion: In this study, we tried to study the normal vasculature of terminal villi by the histological method, which explains about normal physiology of blood circulation in the placenta.

Key Words: Terminal villi, term placenta, blood vessels.

INTRODUCTION

Successful pregnancy depends on the implantation and formation of fetal and maternal blood vessels to supply the increasing demand for nutrition for developing the embryo. Organized angiogenesis is required for optimal nutrient transfer between mother and fetus. Inadequate vessel formation or maintenance in the placenta results in impaired placental growth, malfunction, hypoxia, and fetal malnutrition that may result in fetal death (1).

Development of vessels within the villi undergoes three stages; vasculogenesis, branching angiogenesis and nonbranching angiogenesis. Vasculogenesis includes the formation of blood vessels in the villi, which is initiated by the expression of angiogenic growth factors. In branching angiogenesis existing vessels will sprout and branch, and increase the number of vessels. In nonbranching angiogenesis, exiting villi are converted into terminal villi. Terminal villi form the functional units of the placenta (2). The terminal villi consist of a layer of cytotrophoblast and syncytiotrophoblast with a core of mesoderm with blood vessels. These villi form the placental barrier and transfer nutrients from mother to fetus and waste products from fetus to mother. It also acts as a barrier for pathogens and the maternal immune system (3). The cells of these villi serve as an endocrine organ and synthesize a plethora of hormones, growth factors, and other bioactive products which maintain pregnancy (4). The placenta is a mirror of prenatal

fetal development and examining the placenta after delivery will give a history of prenatal fetal and maternal health (5). The histological examinations of the placenta are frequently used to study the prenatal history of events and in identifying the cause of death of fetus and complications for clinicians and parents (6).

This study has been aimed to see the number of blood vessels/terminal villi in the placenta of 37 - 41 weeks of gestation. This will give an idea about the normal vascular structure of the terminal villi.

MATERIALS AND METHODS

One hundred and fourteen normal placentae were collected immediately after delivery with the consent of the participant. A section of the placenta was taken from the center, fixed in 10% neutral buffered formalin and processed for Hematoxylin & Eosin (H & E) staining. Stained slides were observed under 40X for counting vessels. Randomly 10 villi were selected from the different regions of tissue, and an average of 10 villi vessels was taken for the count and tabulated.

RESULTS

We studied 114 normal placentae, in H & E staining we observed that mean of blood vessels/terminal villi (Figure No. 1) was 6.88 with standard deviation 1.39 and the number of vessels in terminal villi of 37-41 weeks gestation period is shown in Table No. 1. We also observed that the number of vessels/terminal villi was reduced from 37 weeks to 41 (Graph No. 1).

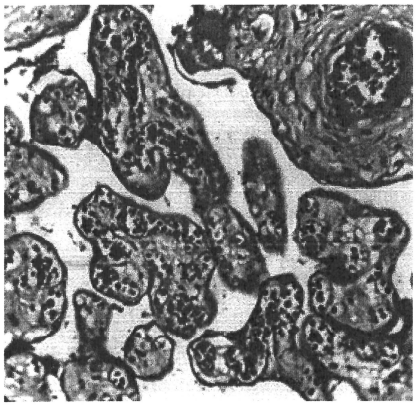
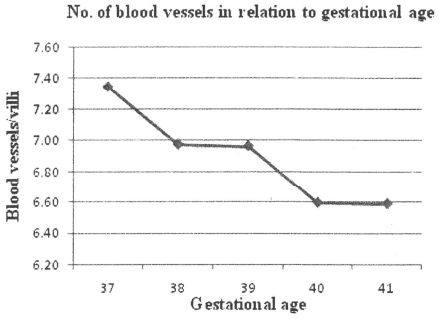


Fig. No.1: Terminal villi (TV) with blood vessels (BV)

Table 1: Mean number of blood vessels / terminal villi in 37 – 41 weeks of gestation period.

Gestational age (GA) in weeks	Number of cases (n)	Mean	SD
37	12	7.35	1.08
38	30	6.98	1.54
39	30	6.96	1.18
40	34	6.60	1.35
41	8	6.71	2.03
Total	**114**	**6.88**	**1.39**

Graph 1: Number of blood vessels / terminal villi in relation to gestational age



DISCUSSION

Terminal villi are produced by the mature intermediate villi by out-bulging of coiled capillaries. The terminal villi development is influenced by the longitudinal growth of mature intermediate villi and its capillaries. The number of terminal villi is produced by the more capillary growth, which exceeds the longitudinal growth of the mature intermediate villi. The capillary growth is influenced by hypoxia. The terminal villi contain only capillaries and sinusoids. The capillary loops of terminal villi arise from the intermediate villi and are connected to the neighboring terminal villi. Thus blood leaving from the terminal villi crosses 3-5 terminal villi. They are arranged parallel to each other. The average vessel diameter of the terminal villi is 12.3 µm, and the length is 3,000-5000 µm (7). There are many angiogenic growth factors identified in the placenta, which influence the formation of terminal villi i.e., non-branching angiogenesis. Among these

VEGF is said to be the potent angiogenic growth factor in the placenta. In branching angiogenesis Vascular Endothelial Growth Factor (VEGF), fms like thyrosine kinase receptor-1 (flt-1), Kinase Domain Receptor (KDR) are increased, and in non-branching angiogenesis, Placental Growth Receptor (PlGF) and flt-1 are increased, while VEGF is decreased (2,8).

In this study, we observed that the mean number of blood vessels/terminal villi of 37-41 weeks of gestational age placenta was 6.88 ± 1.39. The number of the vessel was reduced from 37 to 41 weeks of gestation. In one of the studies on terminal villi of the normal and gestational diabetic placenta, it was observed that the density of blood vessels/unit area was 10.70 ± 4.66 (mm^3) in normal and in diabetic it was 21.76 ± 8.52 (mm^3) / unit area. The increased number of blood capillaries is due to hyperplasia of terminal villi and low oxygen content (9). In normal, the core of terminal villi consists of 1-6 capillaries/ terminal villi (3). In one of the histomorphometric study of terminal villi of normal and pre-eclamptic placentae, it was observed that the vascular density in normal was 25.63 ± 8.88 mm^3 and 22.04 ±8.72 mm^3 per unit area in pre-eclamptic terminal villi (10). Studying normal vasculature will help to understand the placental physiology and pathology.

CONCLUSION

Vessels of the terminal villi are essential areas of gaseous exchange between fetus and mother for the growth of the fetus. In this study, we tried to study the normal vasculature of terminal villi by the histological method, which explains about normal physiology of blood circulation in the placenta.

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ANNEXURE – V - CERTIFICATE OF PAPER PRESENTATION

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9th ANNUAL INTERNATIONAL CONFERENCE of INDIAN ACADEMY OF BIOMEDICAL SCIENCES

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Type of Conference-Multi speciality

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has Participated as Delegate / Delivered an Oral / ~~Poster~~ presentation in the 9th Annual International Conference of Indian Academy of Biomedical Sciences.

He / She is granted 4 CPD credit points by Maharashtra Medical Council.

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Prof. (MS) Syed Mudassar	Prof. Abbas Ali Mehadi	Prof. Bipin M. Tiwale	Dr.R.K.Sharma	Dr.Sandeep S. Kadam	Dr.Shailesh Kore
President, IABS	Secretary, IABS	Organizing Secretary	Organizing Chairman	MMC Observer (MMC/MAO-01898/2018)	MMC Observer (MMC/MAO-00603/2015)