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

Vanitha[1*], Dixit Daksha[2], Sanikop Adarsh[3], Tyagi NK[4] and Bhimalli Shilpa M[5]

[1,2,5] Department of Anatomy, Jawaharlal Nehru Medical College, KLE Academy of Higher Education & Research (KAHER), Belagavi, Karnataka, India.
Department of pathology, Jawaharlal Nehru Medical College, KLE Academy of Higher Education & Research (KAHER), Belagavi, Karnataka, India.
Department of Epidemiology and Biostatistics, Jawaharlal Nehru Medical College, KLE Academy of Higher Education & Research (KAHER), Belagavi, Karnataka, India.

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.

Corresponding Author:

Vanitha

Ph. D. Scholar,

Department of Anatomy, Jawaharlal Nehru Medical College,

KLE Academy of Higher Education & Research (KAHER) Nehru Nagar, Belagavi.

Email-vanithasanjeev@gmail.com. Mobile No.: 8095600731

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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