Original Article

DOI: 10.21276/APALM.2834

Immunohistochemical Study of VEGF in Placenta of Hypertensive Mothers

Sharath Kumar H K*, Chaitra N, Nataraju G and Bharathi M

Department of Pathology, Mysore Medical College and Research Institute

ABSTRACT

Background: Pregnancy is most commonly complicated by Hypertensive disorders. In India, the incidence of gestational hypertension varies from 0.5-1.8%. VEGF is a prime regulator of angiogenesis and overall maintenance of endothelial cell health. This study aims to determine the role of VEGF in placentae of Hypertensive and Normotensive pregnancies by assessing its immunohistochemical expression in Syncytiotrophoblasts.

Methods: The study was conducted in the Department of Pathology in our institute. This is a case-control study which included 50 placentae. Out of which,25 were from Normal mothers and 25 placentae from Hypertensive mothers. Immunohistochemistry for VEGF was performed on tissue section using commercially available monoclonal antibodies. The results were interpreted by evaluating Positivity and Intensity of Immunostaining.

Result: Out of 25 Hypertensive placentae, 22 showed Positivity for VEGF immunostaining. Out of 25 Normotensive placentae, 23 showed Negative results for syncytiotrophoblastic staining of VEGF. The difference in VEGF expression in syncytiotrophoblast of hypertensive and normotensive placentae was statistically significant.

Conclusion: Hypoxia acts as a potent stimulus for induction of VEGF mRNA in an attempt to normalize fetal blood flow and thus VEGF is increased. This results in the notable increase in immunohistochemical expression of VEGF in the syncytiotrophoblasts of hypertensive placenta.

Keywords: *Hypertensive Placenta, Immunohistochemistry, Syncytiotrophoblasts, VEGF*

Introduction

Pregnancy is most commonly complicated by Hypertensive disorders; Pre-Eclampsia and Eclampsia being one of the leading causes of maternofetal morbidities and deaths. [1] In India, the incidence of gestational hypertension varies from 0.5-1.8 %. 5-15% of gestational hypertension leads to mortality. [2]

Physiologically, Endovascular trophoblastic invasion transforms uterine spiral arterioles into low impedence, high calibre capacitance vessels. This is achieved by interactions between various vasodilator and vasoconstrictor factors, and the normal uteroplacental blood flow is achieved by the mitogenic glycoprotein i.e., Vascular Endothelial Growth Factor (VEGF). [3] VEGF is a disulphide linked homodimeric glycoprotein which is a prime regulator of angiogenesis and overall maintenance of endothelial cell health. [4] It has seven isoforms among which VEGF-A and PIGF, which are synthesised by the villous syncytiotrophoblasts, are known to regulate the remodelling of spiral arterioles. [1]

Placenta, under hypoxia and oxidative stress gives rise to Placental Pre-Eclampsia. Poor trophoblastic invasion leads to defective remodelling of maternal uterine spiral arteries and reduces the placental perfusion of oxygen and nutrients to the fetus. This is known as 'Placental Hypoxia Theory'.

This leads to increased sFlt-1, which is an endogenous inhibitor of VEGF, resulting in decreased binding of VEGF-A, and thus causes impaired angiogenesis. Thus Hypoxia potentially induces VEGF Gene expression. [5]

Placenta is a highly complex and fascinating organ, but often discarded at birth. Aberrations of placental function have immediate consequences on outcome of pregnancy and influence the lifelong health of the offspring. These intrapartum aberrations of placental function can be assessed by morphological study of placenta. [6]

Endothelial dysfunction and Vasospasm act as main etiologic factors in Pre-Eclampsia. Although there are various explanations, the mechanism of Endothelial dysfunction in Hypertensive Disorders of Pregnancy is still an enigma. Moreover, there are several studies showing the increased levels of serum VEGF in pregnancies complicated by pre-eclampsia but there are limited number of studies focusing on its expression in placental tissue of hypertensive mothers as compared to uncomplicated pregnancies. This study aims to determine the role of

VEGF in placentae of Hypertensive and Normotensive pregnancies by assessing its Immunohistochemical expression in Syncytiotrophoblasts.

Materials and Methods

The study was conducted in the Department of Pathology, Mysore Medical College and Research Institute, Mysore. This is a case- control study which included 50 placentae. Out of which, 25 were from Normal mothers and 25 placentae from Hypertensive mothers.

The Medical Records of all the patients were reviewed and the relevant clinical details were obtained. Resected and biopsy specimens were received in the department of pathology. In every case the standard protocol for surgical grossing of resected specimens was followed. After conventional processing, paraffin sections of 5µm thickness were stained by haematoxylin and eosin for histopathological study. In addition, 4µm sections were cut from a paraffin block of placental tissue and taken on the glass slide coated with adhesive (silane) for immunohistochemistry to detect expression of Vascular Endothelial Growth Factor (VEGF)

Immunohistochemistry for VEGF performed on tissue section using commercially available monoclonal antibodies. Heat antigen retrieval were done by using pressure cooker and standard immunohistochemistry procedure were performed according to the manufacturer's instructions.

The results were interpreted by evaluating the Positivity and Intensity of Immunostaining of Syncytiotrophoblasts for VEGF marker. The Intensity of positive cells was scored as Weak, Moderate or Strong.

Statistical Data analysis will be done using SPSS (Statistical Package for Social Sciences) Software Version 20.0. Descriptive statistics like frequencies and percentages will be calculated for VEGF expression. Chi square test were used as inferential test. Any p value < 0.05 was considered statistically significant.

Result

The Immunostaining of VEGF was evaluated in syncytiotrophoblast cells of Normotensive and Hypertensive placentae. The Positivity and Intensity of staining was evaluated.

Out of 25 Hypertensive placentae, 22 showed Positivity for VEGF immunostaining (88 %) (Figure 1 and 2). Majority of the cases showed moderate to strong staining. Moderate staining was seen in 7/22 VEGF positive cases accounting for 28%. Strong immunostaining was observed in 15/22 VEGF positive cases accounting for 60%. None of the positive cases showed weak staining.

Out of 25 Normotensive placentae, 23 showed Negative results for syncytiotrophoblastic staining of VEGF. Thus, 92% of normotensive placenta showed negative immunostaining.

The results are tabulated in Table 1.

The difference in VEGF expression in syncytiotrophoblast of placentae of hypertensive and normotensive pregnancy was statistically significant. (p value < 0.05)

Discussion

Normal uteroplacental blood flow is maintained by complex interaction of regulatory factors. Growth factors have predominant role in this mechanism. Chronic hypoxia acts as main stimulus for Endothelial dysfunction and thus ineffective maternofetal circulation and insufficient supply of blood and nutrients. This may lead to defective function of growth factors namely, VEGF and PIGF. In this study we have mainly focused on the immunohistochemical expression of VEGF in the placental tissue.

Azliana AF et al., showed that VEGF was significantly increased in syncytiotrophoblasts in hypertensive group, but not in cytotrophoblasts, decidual cells, fetal and maternal endothelial cells. Our study also showed moderate to strong staining of VEGF in syncytiotrophoblasts of majority of the hypertensive placentae.[1]

Table 1: showing positivity and intensity of VEGF immunoreactivity.

	Hypertensive Placentae (25)		Normotensive Placentae (25)	
	Positive immunostaining	Negative immunostaining	Positive immunostaining	Negative immunostaining
Strong	15 (60%)	-	-	-
Moderate	7 (28%)	-	2 (8%)	-
Weak	-	-	-	-
Total	**22 (88%)**	**3(12%)**	**2 (8%)**	**23 (92%)**

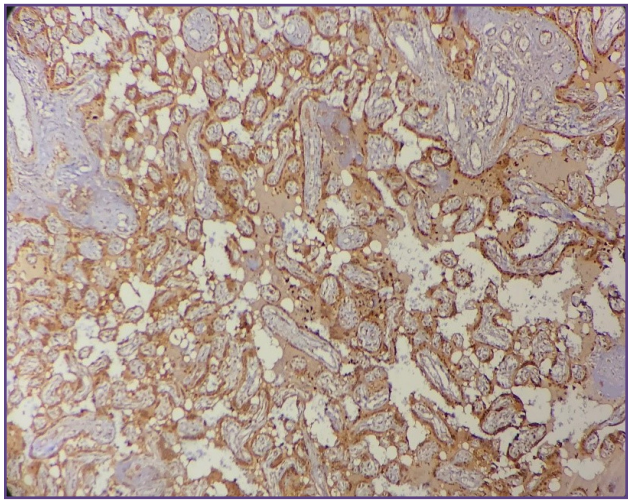

Fig. 1: VEGF in hypertensive placenta showing positive staining. Immunohistochemical stain; 10x.

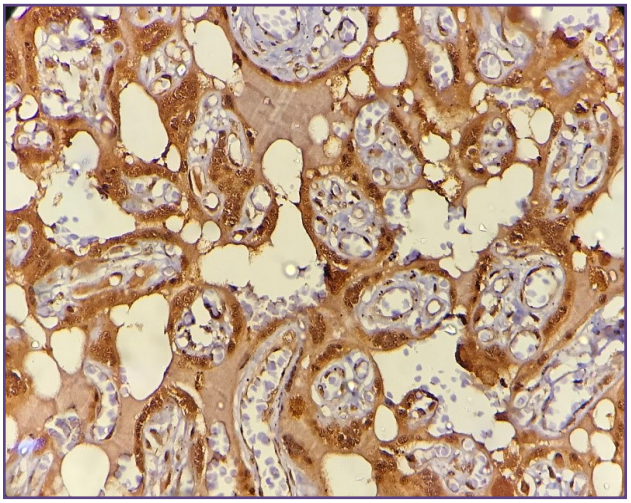

Fig. 2: Strong staining of VEGF in hypertensive placenta Immunohistochemical stain; 40x.

Yelumalai S et al., conducted the study on plasma levels of angiogenic factors, namely sFlt-1, VEGF and PIGF in normal pregnant women, pregnant with PIH and pre-eclamptic patients. There was a huge attention drawn to role of VEGF. In their study, the plasma levels of VEGF were significantly lower in the pre-eclamptic group. This explains the increased expression of receptors of pro-angiogenic factor, VEGF at the placental level to restore the angiogenesis. [7]

The study conducted by Emel Kurtoglu et al., concluded that VEGF was increased in placentas of severe pre-eclamptic patients as compared to mild pre-eclampsia and the controls. In our study, we have not categorised the cases into mild, moderate or severe, but in general, there is intensive staining of VEGF as compared to the control group. [8]

E Sgambati et al., proved that the increase in placental VEGF is to restore the uterine blood flow towards normal and thus shows different expression of VEGF according to different degrees of clinical severity. Moderate to strong staining observed in our study might correspond to the severity of hypertension. [9]

The study conducted by Maynard SE et al., showed that the sFlt-1 which is an antagonist of VEGF and PIGF is upregulated in pre-eclampsia. Thus circulating free VEGF and PIGF are reduced, resulting in endothelial dysfunction in vitro. The hypothesis that placental hypoxia induces VEGF levels in an attempt to compensate the decreased levels of free VEGF and PIGF is thus supported. [12]

Thus, the imbalance in the angiogenic factors has a key role in endothelial dysfunction, which leads to pre-eclampsia. This study draws a substantial interest on the role of VEGF in this condition and VEGF can be used as a marker of Hypertensive disorder of pregnancy for the early detection and effective treatment.

Conclusion

Hypoxia acts as a potent stimulus for induction of VEGF mRNA in an attempt to normalize fetal blood flow and thus the growth factor VEGF is increased, which is a compensatory mechanism. This results in the notable increase in immunohistochemical expression of VEGF in the syncytiotrophoblasts of hypertensive placenta. It is a convenient and reproducible method of assessing the proliferation of syncytiotrophoblasts and thus it can be used as marker of Hypertensive disorder of pregnancy.

Acknowledgements

I thank all the technicians of our institution for the technical help provided. I thank Dr Bharathi M, Head of the department of pathology for the constant support. I would express my sincere gratitude to the Department of Obstetrics for being considerate during the entire study period.

Funding

None

Competing Interests

None declared

References

1. Azliana AF, Zainul-Rashid MR, Chandramaya SF, Farouk WI, Nurwardah A, Wong YP, et al. Vascular endothelial growth factor expression in placenta of hypertensive disorder in pregnancy. Indian J Pathol Microbiol 2017; 60:515-20.

2. Thobbi VA, Anwar A. A study of maternal morbidity and mortality due to Pre eclampsia and eclampsia. Al Ameen J Med Sci 2017;10(3):174-179.
3. Dutta DC. Textbook of obstetrics. 7th edition. New Delhi: Jaypee Brothers Medical Publishers;2013.
4. Klagsbrun M, D'Amore PA. Vascular Endothelial Growth Factor and its Receptors. Cytokine & Growth Factor Reviews 1996; 7(3):259-270.
5. Redman WC, Sargent LL. Latest Advances in Understanding Preeclampsia. Science 2005; 308:1592-1594
6. Baergen RN. Manual of Benirschke and Kaufmann's Pathology of the Human Placenta. New York: Springer;2005.
7. Yelumalai S, Muniandy S, Zawiah Omar S, Qvist R. Pregnancy-induced hypertension and preeclampsia: Levels of angiogenic factors in Malaysian women. J Clin Biochem Nutr 2010; 47:191-7.
8. Kurtoglu E, Altunkaynak BZ, Aydin I, Ozdemir AZ, Altun G, Kokcu A, Kaplan S. Role of vascular endothelial growth factor and placental growth factor expression on placenta structure in pre-eclamptic pregnancy. J Obstet Gynaecol Res 2015; 41(10):1533-1540.
9. Sgambati E, Marini M, Thyrion GDZ, Parretti E, Mello G, Orlando C et al., VEGF expression in the placenta from pregnancies complicated by hypertensive disorders. Br J Obstet Gynaecol 2004; 111:564-570
10. Walker JJ. Pre-eclampsia. Lancet 2000; 356:1260-5.
11. Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. Annu Rev Med 2008; 59:61-78.
12. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003; 111:649-58.

***Corresponding author:**
Dr. Sharath Kumar H K, Department of Pathology, K R Hospital, Irwin road, Mysuru, Karnataka- 570001
Phone: +91 9743813949
Email: sharutanu1748@gmail.com

Financial or other Competing Interests: None.

Date of Submission : 22-03-2020
Date of Acceptance : 07-08-2020
Date of Publication : 30-09-2020