ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Prospective Study On Expression Of VGEF In Placentas Of Pregnant Mothers With Hypertension Of Different Severity By Immunohistochemistry.

P Sindhuja[1]*, and Keerthini Ganesan[2].

[1]Assistant Professor, Department Of Pathology, Regional Institute Of Ophthalmology And Government Ophthalmic Hospital, Chennai, Tamil Nadu, India.
[2]Assistant Professor, Department of Pathology, ACS Medical College & Hospital, Poonamallee High Rd, Velappanchavadi, Chennai, Tamil Nadu, India.

ABSTRACT

Pregnancy induced hypertension syndrome (PIH) is a high-incidence disease of pregnant women that can damage the heart, brain, kidney and other organs. PIH reduces uterine and placental blood perfusion and can severely impair foetal survival. The possibility of risk occurrence increases in a PIH patient suffering from congestive heart failure. VEGF, a cytokine on the surface of vascular endothelial cells, macrophages and nurse cells, is involved in vascular formation and reconstruction; VEGF combines with a specific receptor to increase Vaso permeability. VEGF is significant in the maintenance of vascular integrity and normal permeability and has a critical regulatory function in pathological physiology associated with hyperplasia of vascular endothelial cells. To determine the expression of VEGF by immunohistochemistry in placental biopsies in pregnant mothers with hypertensive disorders of vaired clinical severity and compare with normal controls. VEGF level in the pregnancy group was significantly higher than in the normal group, and the difference between these two groups was significant ($P < 0.001$). In the pregnancy group, VEGF reached the maximum level at the metaphase stage of pregnancy and started to decrease at advanced pregnancy. VEGF level in the PIH group was significantly lower than in the pregnancy group at advanced pregnancy ($P < 0.01$), and VEGF level significantly and gradually decreased with PIH aggravation ($P < 0.05$).The significant decrease of VEGF level after pregnancy was possibly an important factor of PIH pathogenesis.

Keywords: Cardiovascular, Hypertension, Pregnancy complications, Vascular endothelial growth factor

https://doi.org/10.33887/rjpbcs/2023.14.6.48

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Hypertension in pregnancy predisposes women to cerebral hemorrhage. Anecdotal reports imply that there may also be an increased risk of myocardial infarctions developing in these women [1]. Preeclampsia is a severe form of pregnancy-induced hypertension affecting 7% to 10% of all first-time pregnancies. The maternal syndrome is characterized by an elevated blood pressure, proteinuria, and edema. Recent studies showed that women with a history of preeclampsia have higher circulating concentrations of fasting insulin, lipid and coagulation factors postpartum, and that there is a specific defect in endothelial-dependent vascular function [2]. The cause of preeclampsia remains unknown. However, the placenta is involved, because preeclampsia can occur in hydatidiform mole when placental tissue alone is present; the delivery of the placenta is the only known cure for preeclampsia. Insufficient adaptation of the decidual and intramyometrial portions of the spiral arterioles in preeclampsia results in reduced utero-placental blood flow, leading to local placental hypoxia [3].

Vascular endothelial growth factor (VEGF) is upregulated by hypoxia and is a potent vascular-protective and angiogenic factor in the placenta [4]. VEGF mediates its signal via two tyrosine kinase receptors, VEGF receptor-1 (VEGFR-1/Flt-1) and VEGFR-2 (KDR/Flk-1). VEGFR-1 can also be expressed as a soluble protein and is generated by alternative splicing of the fms-like tyrosine kinase (*flt-1*) gene [5]. Soluble VEGFR-1 (sVEGFR-1/sFlt-1) mRNA is expressed at high levels in the placenta and is produced by villous and extravillous trophoblasts [6]. VEGF-binding protein in amniotic fluid were higher in women with preeclampsia compared with those with normal pregnancy, and this protein is increased in preeclamptic placenta [7]. Soluble VEGFR-1 has strong antagonistic activity as it binds to all isoforms of VEGF and placenta growth factor (PlGF), and it can also form dominant-negative complexes with mitogenitically competent full-length VEGFR [8]. More recent studies showed that serum levels of sVEGFR-1 in women with preeclampsia were elevated [9] VEGF is mainly expressed on the surface of placental syncytiotrophoblast cells and invasive chorionic trophoblast cells during pregnancy; VEGF is particularly expressed at the vascular bud site at early pregnancy, during which syncytiotrophoblast cells are abundant. VEGF has an important function in vascular endothelial damage, and various growth factors have an important function in disease [10].

MATERIALS AND METHODS

This prospective study was conducted in the year 2017-2017 at institute of obstetrics and gynaecology and govt. Hospital for women and children, Chennai, Tamil Nadu, India. Pregnant women with hypertension attending obstetrics and gynaecology department are included for the study. Seventy-five patients were included in our study with 34 patients in normotensive group and 41 patients in the hypertensive group.

Inclusion Criteria

BP ≥ 140/90 mm Hg diagnosed first during the present pregnancy (or) BP ≥140/90 mm Hg after 20 weeks of gestation on two consecutive measurements 4 hours apart. Proteinuria ≥ 300mg/ 24 hr urine sample (or) ≥ 1+ on dipstick on random urine sample .

Exclusion Criteria

Pregnant women with complications other than hypertension. Hypertension due to underlying renal disease. The study was conducted in accordance with the guidelines approved by the institutional review board of our Institution. The study population was divided into two groups namely normotensive and hypertensive patients. The hypertensive groups patients were further divided into two groups of mild (BP ≥140/90 mm Hg with proteinuria) and severe (BP ≥ 160/100 mm Hg with proteinuria). The placental specimens were preserved in 10% neutral buffered formalin solution. Informed consent was obtained in all patients and clinical data were obtained from the case notes. Specimen for immunohistochemistry was secured from the insertion point of umbilical cord. Three-to-four-micron sections were taken from each of the blocks using Leica 2125RTS microtome. The sections were mounted on the H &E labelled slides. The slides were then stained by Harris haematoxylin and Eosin Staining. Procedure is explained in ANNEXURE - I. When the slides were dry, they were stored in a tray according to study numbers. The cases were evaluated by the principal investigator. This information was recorded on the data sheet. Two-to-four-micron sections were cut from each of the study blocks and mounted on two Pol y - L- lysine treated slides

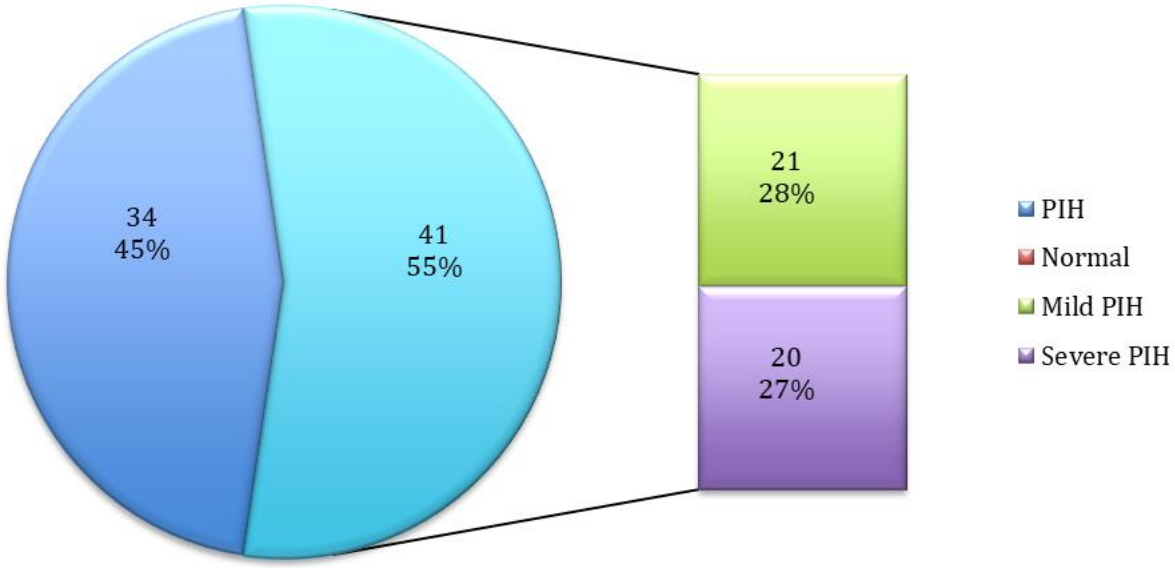
well labelled with lab number and t ype of test. When dried, they were stored serially in a tray and processed in different batches. One positive and one negative control were included in each batch and were therefore subjected to the same test conditions as the study cases. Manual immunostaining procedure was performed after mounting the sections on pol y – L–lysine treated slides. The microscopic estimation was done using an Olympus microscope under 40 X magnification. Positivity and intensity were evaluated on syncytiotrophoblast, syncytial knots, fetal endothelial and decidual cells. Intensity of positive results were graded as Negative : 0,Weak : 1+,Moderate : 2+,Strong : 3+The observations were made by independent observers who were unaware of the patient clinical details. These are aggregates of multiple nuclei at the surface of terminal villi. These knots invade the uterine wall to nourish the growing embryo. In histology, it appears as multinucleated cells. The number of knots were counted in three high power fields and an average number was tabulated.

Statistical Methods

Continuous variables are represented in mean, median, mode and standard deviation. Categorical variables are represented in frequencies and percentages. Pie-charts and bar diagrams are used as appropriate. When a Categorical Variable is associated with a categorical variable, the variables are represented in both by tables and bar diagrams. For test of significance, chi-square test is used. Fisher's exact test is used when more than 20% of the cell values have expected cell value less than 5. When a Continuous variable is associated with the categorical variables such as patient groups independent t test is used after checking for normality. Otherwise non parametric tests were used.P-values less than 0.05 were considered statistically significant. Data was entered in MS excel sheet and analysed using SPSS software version 16.

RESULTS

Graph 1: Distribution of subjects between two groups:

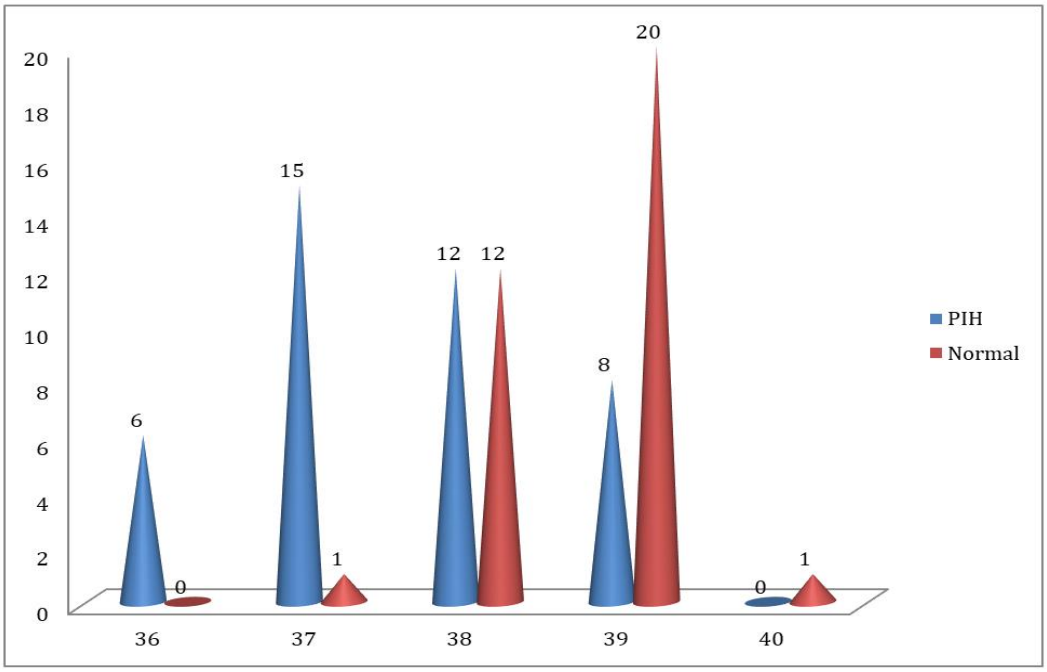


Totally 75 subjects were enrolled in the study, out of which 41 (55%) were hypertensive disorder or pregnancy induced hypertension (PIH) cases and 34 (45%) were normal subjects without any complications. Among the subjects with PIH, 21 subjects had mild PIH and 20 subjects had severe PIH.

The figure shows the distribution of population in which 41 (55%) were hypertensive disorder or pregnancy induced hypertension (PIH) cases and 34 (45%) were normal subjects without any complications. Among the subjects with PIH, 21 (51.22%) subjects had mild PIH and 20 (48.78%) subjects had severe PIH.

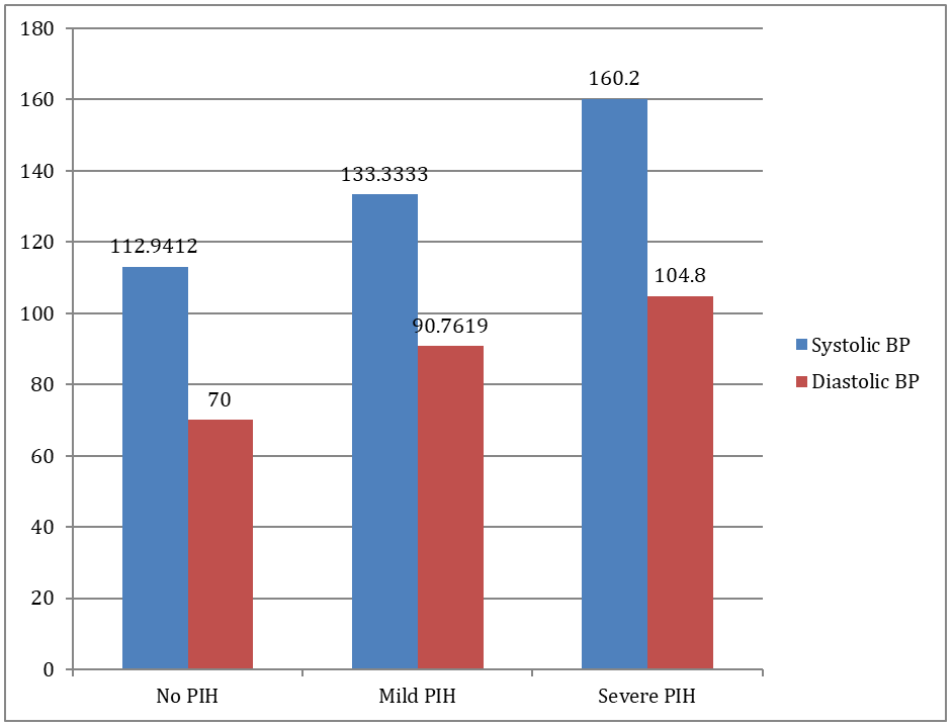
ISSN: 0975-8585

Graph 2: Gestational age distribution among the population



The figure shows that maximum number of subjects 28(37.33%) belonged to 39 weeks gestation. Also 24 (32%) and 16(21.3%) subjects belonged to 38 weeks and 37 weeks respectively. The mean (SD) gestational age of PIH was 37.96(088) weeks and that of normal group were 38.97(0.59). The mean gestational age of the population was 38.41(0.91) weeks.

Graph 3: Blood pressure measurements among the study population



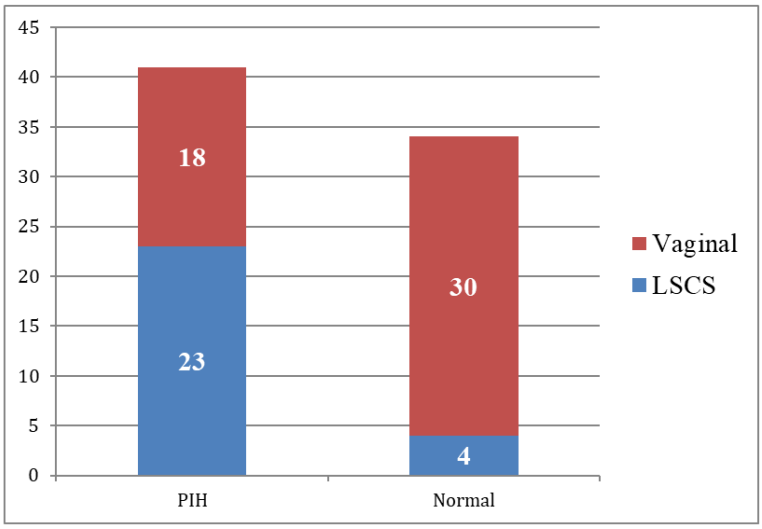
The graph 3 shows the mean (SD) systolic and diastolic BP of the total population were 131.25(21.027) mm Hg and 85.093(15.06) mm Hg respectively. The systolic BP ranges from 110 to 174 mm Hg and diastolic BP ranges from 70 to 110 mmHg. The mean (SD) systolic and diastolic BP of mild PIH

ISSN: 0975-8585

subjects were 133.33(10.64) mm H and 90.76(2.48) mm Hg respectively. The systolic BP of the mild PIH subjects ranges from 120 to 150 mm Hg and that of diastolic BP ranges from 90 to 100 mm Hg. The mean (SD) systolic and diastolic BP of severe PIH subjects were 160.20(8.87) mm H and 104.80(5.00) mm Hg respectively. The systolic BP of the severe PIH subjects ranges from 140 to 174 mm Hg and that of diastolic BP ranges from 100 to 110 mm Hg.

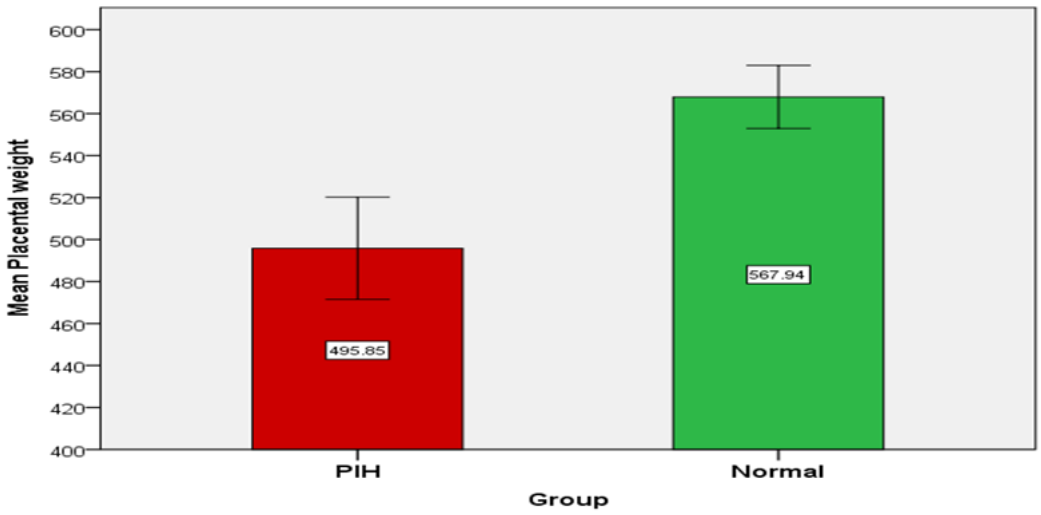
The figure shows the mean (SD) systolic and diastolic BP of the total population were 131.25(21.027) mm Hg and 85.093(15.06) mm Hg respectively. The systolic BP ranges from 110 to 174 mm Hg and diastolic BP ranges from 70 to 110 mmHg. The mean (SD) systolic and diastolic BP of mild PIH subjects were 133.33(10.64) mm H and 90.76(2.48) mm Hg respectively. The systolic BP of the mild PIH subjects ranges from 120 to 150 mm Hg and that of diastolic BP ranges from 90 to 100 mm Hg. The mean (SD) systolic and diastolic BP of severe PIH subjects were 160.20(8.87) mm H and 104.80(5.00) mm Hg respectively. The systolic BP of the severe PIH subjects ranges from 140 to 174 mm Hg and that of diastolic BP ranges from 100 to 110 mm Hg.

Graph 4: Mode of delivery among the study population



The figure shows that majority of the subjects 48(64%) had vaginal delivery among the study population. Also it shows that majority 23(56.09%) of the PIH subjects had LSCS.

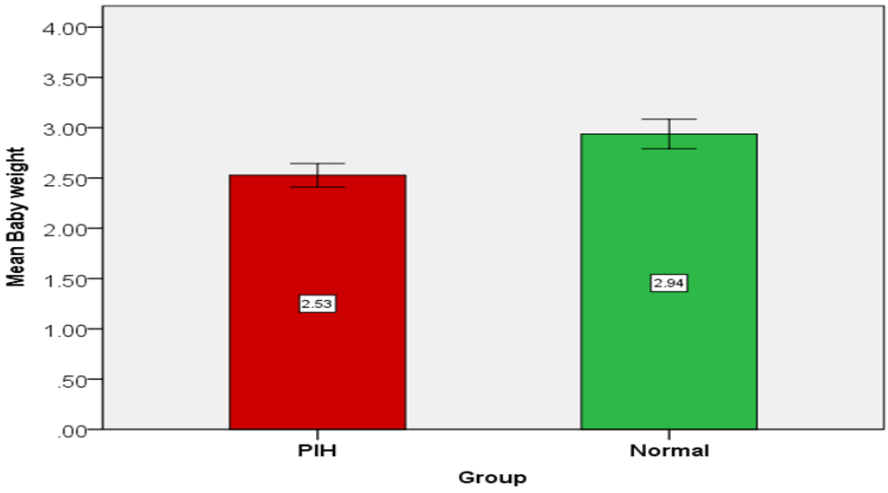
Graph 5: Placental weight among the population



The graph shows that mean (SD) placental weights among the normal subjects 567.94(43.05) gm were higher compared to that of PIH subjects.

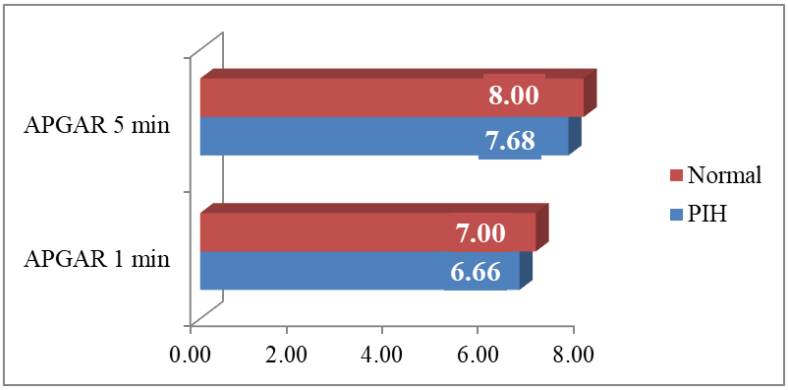
ISSN: 0975-8585

Graph 6: Birth weight of the baby among the population



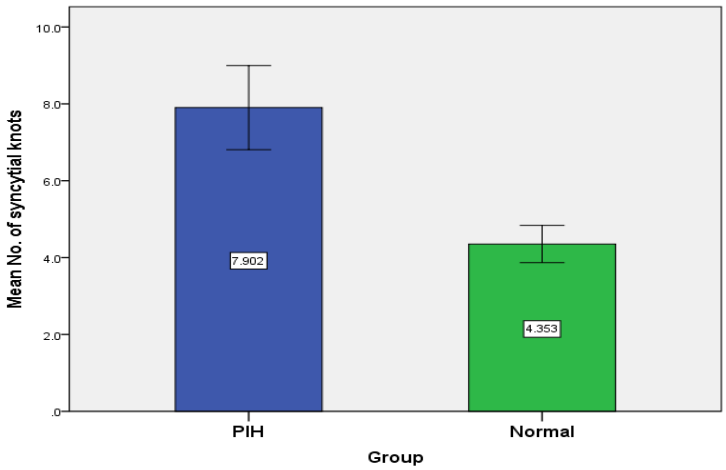
The figure shows that mean (SD) weight of the baby among normal subjects 2.94(0.41) kg were more compared to PIH cases 2.53 (0.37) kg.

Graph 7: APGAR among the study population for 1 minute and 5 minutes



The graph shows that mean (SD) APGAR at 5 minute among the normal subjects 8.00(0.00) were more compared to the PIH cases7.68 (0.61). Also mean (SD) APGAR at 1 minute among the normal subjects 7.00(0.00) were more compared to the PIH cases 6.66(0.66).

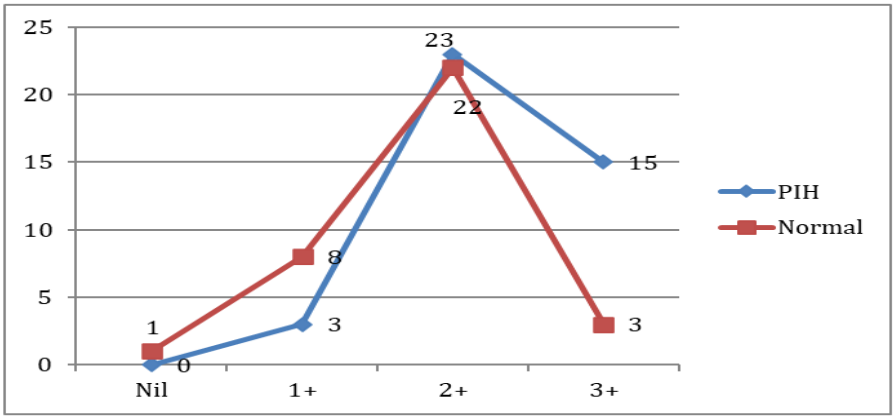
Graph 8: Distribution of syncytial knots among the study population



ISSN: 0975-8585

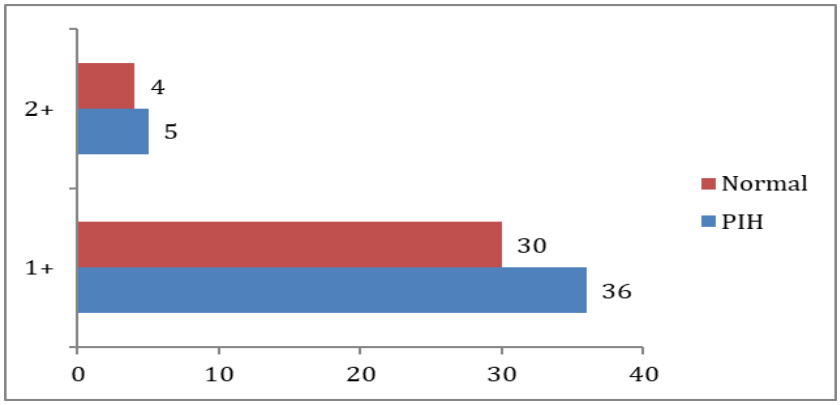
The graph shows that mean(SD) of the syncytial knot distribution among the PIH group 7.90(3.47) was more compared to that of normal subjects 4.35(1.39).

Graph 8: Intensity of VEGF expression in syncytiotrophoblasts among the study population



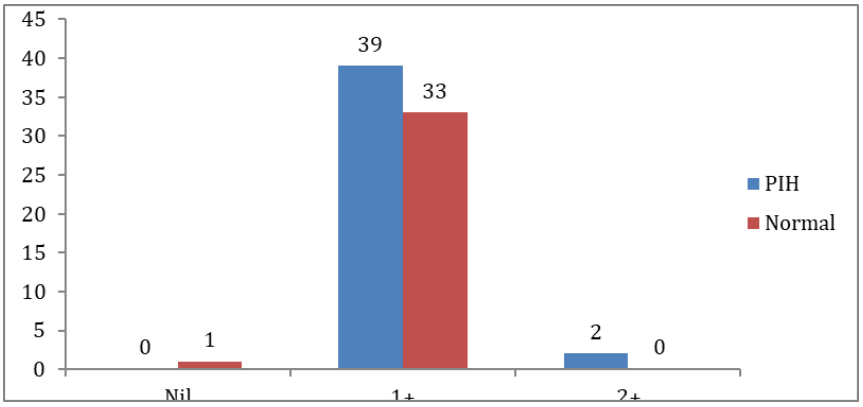
The figure lie graph shows that majority of the 15(83.33%) PIH subjects were having 3+ intensity whereas 3(16.64%) normal subjects only had 3+ intensity of VEGF.

Graph 9: Intensity of VEGF staining in foetal endothelial cells among the study population



The figure shows that majority of the PIH subjects 36(54.54%) had 1+ presence of fetal cells compared to normal subjects 30(45.45%).

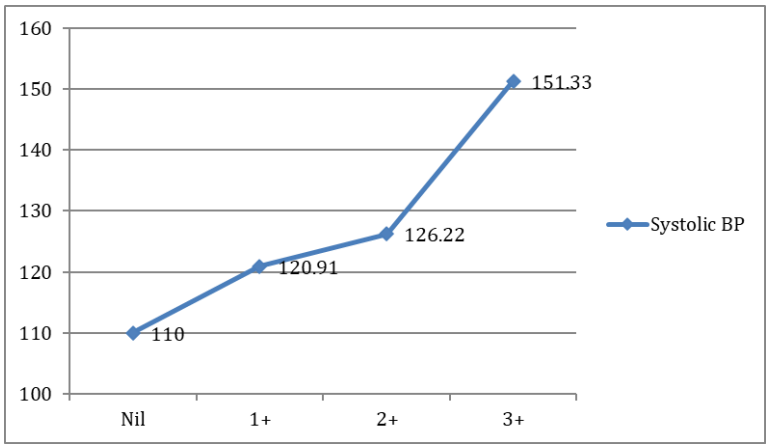
Graph 10: Intensity of VEGF staining in decidual cells among the study population



The figure shows that majority of the PIH subjects 39(54.16%) had 1+ presence of decidual cells compared to normal subjects 33(45.83%).

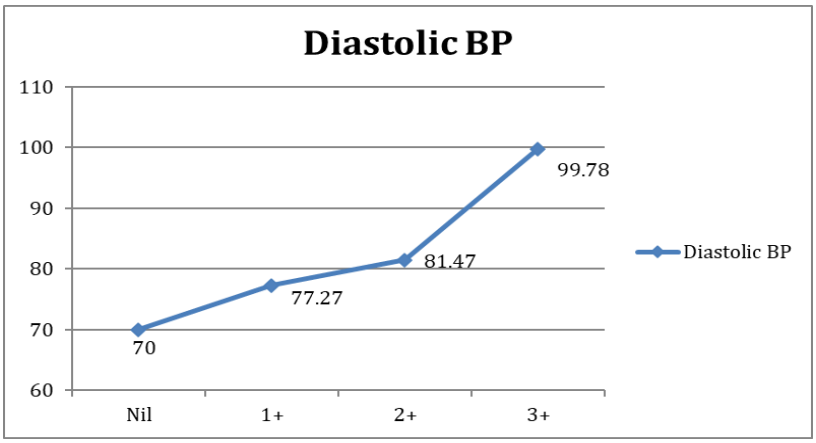
ISSN: 0975-8585

Graph 11: VEGF and systolic BP



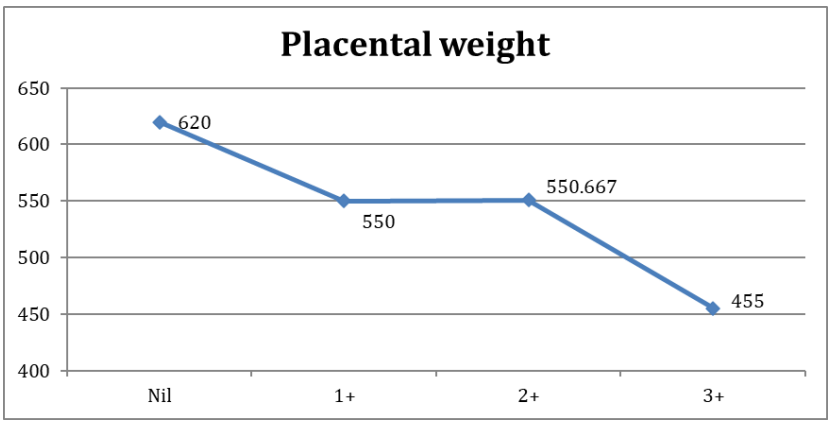
The figure shows that subjects who had 3+ VEGF had a mean (SD) systolic BP value of 151.33(20.21) mm Hg.

Graph 12: VEGF and diastolic BP



The figure shows that subjects who had 3+ VEGF had a mean (SD) diastolic BP value of 99.78(14.42) mm Hg.

Graph 13: VEGF and placental weight



The figure shows that as VEGF intensity increases the mean placental weight decreases, particularly in +3 intensity mean (SD) placental weight was455(82.05) gm.

ISSN: 0975-8585

DISCUSSION

In our study 75 subjects were enrolled in the study, out of which 41 (55%) were hypertensive disorder or pregnancy induced hypertension (PIH) cases and 34 (45%) were normal subjects without any complications. Among the subjects with PIH, 21 (51.22%) subjects had mild PIH and 20 (48.78%) subjects had severe PIH. In the study age of the subject lies between 20 and 34 years. In our study maximum 32 (42.67%) subjects belong to primi parity among the population. Among them 15 (46.87%) were diagnosed with PIH. Also second gravidas were 41(54.66%) among the population with G2A1 20(48.78%) and G2P1L1 21(51.22%).In the study maximum number of subjects 28(37.33%) belonged to 39 weeks gestation. Also 24 (32%) and 16(21.3%) subjects belonged to 38 weeks and 37 weeks respectively [11]. The mean (SD) gestational age of PIH was 37.96(088) weeks and that of normal group were 38.97(0.59). The mean gestational age of the population was 38.41(0.91) weeks.In the study the mean (SD) systolic and diastolic BP of the total population were 131.25(21.027) mm Hg and 85.093(15.06) mm Hg respectively. The systolic BP ranges from 110 to 174 mm Hg and diastolic BP ranges from 70 to 110 mmHg. The mean (SD) systolic and diastolic BP of mild PIH subjects were 133.33(10.64) mm H and 90.76(2.48) mm Hg respectively. The systolic BP of the mild PIH subjects ranges from 120 to 150 mm Hg and that of diastolic BP ranges from 90 to 100 mm Hg. The mean (SD) systolic and diastolic BP of severe PIH subjects were 160.20(8.87) mm H and 104.80(5.00) mm Hg respectively. The systolic BP of the severe PIH subjects ranges from 140 to 174 mm Hg and that of diastolic BP ranges from 100 to 110 mm Hg. In our study majority of the subjects 48(64%) had vaginal delivery among the study population. Also, it shows that majority 23(56.09%) of the PIH subjects had LSCS. The mean (SD) placental weights among the normal subjects 567.94(43.05) gm were higher compared to that of PIH subjects 495.85(77.20) gm. The mean (SD) weight of the baby among normal subjects 2.94(0.41) kg were more compared to PIH cases 2.53 (0.37) kg. The mean (SD) APGAR at 5 minute among the normal subjects 8.00(0.00) were more compared to the PIH cases7.68 (0.61). Also mean (SD) APGAR at 1 minute among the normal subjects 7.00(0.00) were more compared to the PIH cases 6.66(0.66) [12]. The mean (SD) of the syncytial knot distribution among the PIH group 7.90(3.47) was more compared to that of normal subjects 4.35(1.39). In our study majority of the 15(83.33%) PIH subjects were having 3+ intensity whereas 3(16.64%) normal subjects only had 3+ intensity of VEGF. In the study majority of the PIH subjects 36(54.54%) had 1+ (weak) expression of fetal endothelial cells which was similar to normal subjects 30(45.45%). Also majority of the PIH subjects 39(54.16%) had 1+ presence of decidual cells which was comparable to normal subjects 33(45.83%) [13]. Immune-histo-chemical evaluation of VEGF in placenta biopsies of preeclampsia complicated pregnancies. The VEGF expression was significantly higher in biopsies of the placenta of patients with pre-eclampsia compared to that of controls. (Mean 271.2, SD 22.65 vs. Mean 201.9, SD 12.33, with P-value=0.000). Placental growth factor, vascular endothelial growth factor, and hypoxia-inducible factor-1α in the placentas of pre-eclamptic women of Sudan [14]. They found that VEGF was significantly expressed more in the placentas of the pre-eclampsia: in 32%, 17.6%, and 14.9% (p value = 0.020) of the placentas of women with severe or mild pre-eclampsia and in controls, respectively. Expression of VEGF in the placenta from pregnancies complicated by hypertension in Italy [15]. Immune reactivity of VEGF was noticeable in all the components of the placenta in the gestational hypertension cases as in the controls. In the cases with pre-eclampsia and pre-eclampsia with HELLP syndrome, some components of the placenta were not immune reactive [16]. The role of vascular endothelial growth factor and placental growth factor expression during the pre-eclampsia on pregnancy on placenta structure. They found that there was no significantly difference between the total volumes of the villi among the groups ($P > 0.05$). On the contrary, on immunohistochemistry, there was significant difference in the numerical density of VEGF-positive cells in severe pre-eclampsia compared to the control and mild pre-eclampsia groups (P value < 0.05) [17]. Expressions of VEGF-A and VEGFR-2 in placentae from pregnant women with GDM. They concluded that there were reduced expressions of VEGFA and VEGFR-2 mRNAs and protein in placental tissues affected with GDM, suggesting that maternal GDM disturbs the pathophysiological function of placentas. the vascular endothelial growth factor ligands and receptors that by using a combination of in situ and in vitro analyses and concluded that the regulation of human cyto-trophoblast survival are dysregulated in severe preeclampsia and haemolysis, raised liver enzymes, and reduced platelets syndrome [18]. VEGF and its expression of a subset of family members is dysregulated in severe forms of preeclampsia. Expression of the VEGF family mRNA in placenta of adverse pregnancy outcomes and concluded that expression of the VEGF family of angiogenic growth factor mRNA in the placenta is decreased in hypertensive disorders of the gestation, SGA and in pre-term births. serum levels of angiogenic growth factors vascular endothelial growth factor (VEGF), placental growth factor (PIGF) and angiogenin in women with pre–eclampsia and matched controls [19]. They concluded that reduced serum levels of VEGF and PIGF characterize, and consequently appear to be of significance during (the improvement of), pre-eclampsia [20].

CONCLUSION

The mean (SD) of the syncytial knot distribution among the PIH group 7.90(3.47) was more compared to that of normal subjects 4.35(1.39). In our study majority of the 15(83.33%) PIH subjects were having 3+ intensity whereas 3(16.64%) normal subjects only had 3+ intensity of VEGF. In the study majority of the PIH subjects 36(54.54%) had weak staining (1+) of fetal endothelial cells which was comparable to normal subjects 30(45.45%). Also majority of the PIH subjects 39(54.16%) had weak staining (1+) of decidual cells which was comparable to normal subjects 33(45.83%).

REFERENCES

[1] Ahokas RA, McKinney ET. Development and Physiology of the Placenta and Membranes. Glob Libr Women's Med :2009.

[2] Burton GJ, Jauniaux E. Development of the human placenta and fetal heart: Synergic or independent? [Internet]. Vol. 9, Frontiers in Physiology. Frontiers Media SA; 2018

[3] Burton GJ, Fowden AL. The placenta: A multifaceted, transient organ [Internet]. Vol. 370, Philosophical Transactions of the Royal Society B: Biological Sciences. The Royal Society; 2015.

[4] Ilekis J V., Tsilou E, Fisher S, Abrahams VM, Soares MJ, Cross JC, et al. Placental origins of adverse pregnancy outcomes: potential molecular targets: an Executive Workshop Summary of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. In: American Journal of Obstetrics and Gynecology 2016

[5] Placental Bed Disorders: Basic Science and its Translation to Obstetrics - Google Books :2018.

[6] Lyall F. Development of the utero-placental circulation: The role of carbon monoxide and nitric oxide in trophoblast invasion and spiral artery transformation. Microsc Res Tech 2003.

[7] Redman CW, Sargent IL. Latest Advances in Understanding Preeclampsia. Science 2005

[8] Walker JJ. Pre-eclampsia. Lancet [Internet]. 2000 Oct 7 [cited 2018 Nov 27];356(9237):1260–5.

[9] 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.

[10] Durig P, Ferrier C, Schneider H. [Hypertensive disorders in pregnancy]. Ther Umschau 1999.

[11] Tannetta D, Sargent I. Placental disease and the maternal syndrome of preeclampsia: missing links? Curr Hypertens Rep 2013 15(6):590–9.

[12] Baumfeld Y, Herskovitz R, Niv ZB, Mastrolia SA, Weintraub AY. Placenta associated pregnancy complications in pregnancies complicated with placenta previa. Taiwan J Obstet Gynecol 2017 56(3):331–5.

[13] Roberts DJ, Post MD. The placenta in pre-eclampsia and intrauterine growth restriction. Journal of Clinical Pathology 2008;61:1254–60.

[14] Lyall F, Bulmer JN, Duffie E, Cousins F, Theriault A, Robson SC. Human trophoblast invasion and spiral artery transformation: the role of PECAM-1 in normal pregnancy, preeclampsia, and fetal growth restriction. Am J Pathol 2001;158(5):1713–21.

[15] Romero R, Chaiworapongsa T. Preeclampsia: a link between trophoblast dysregulation and an antiangiogenic state. J Clin Invest 2013;123(7):2775–7.

[16] Eswarappa SM, Potdar AA, Koch WJ, Fan Y, Vasu K, Lindner D, et al. Programmed translational readthrough generates antiangiogenic VEGF-Ax. Cell 2014;157(7):1605–18.

[17] Niu G, Chen X. Vascular endothelial growth factor as an anti-angiogenic target for cancer therapy. Curr Drug Targets 2010; 11(8):1000–17.

[18] Nicosia RF. What is the role of vascular endothelial growth factor-related molecules in tumor angiogenesis? Am J Pathol 1998 ;153(1):11–6.

[19] Ferrara N. Vascular Endothelial Growth Factor: Basic Science and Clinical Progress. Endocr Rev 2004;25(4):581–611.

[20] Palmer K. Assessing the circulating placental-specific anti-angiogenic protein sFLT-1 e15a in preeclampsia. In: Methods in Molecular Biology p. 27–37.