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Original Research Paper

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CLINICOPATHOLOGICAL AND RADIOLOGICAL CHANGES IN HIGH RISK PREGNANCIES AND GENETIC ANALYSIS

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ABSTRACT

Introduction: Pre-eclampsia (PE) is a pregnancyspecific syndrome that can affect virtually every organ system. It affects 5-10% of pregnancies worldwide and 4.6% of pregnancies in India. The present study was planned to study clinical picture, Radiological pathological and Genetic factor in Pregnancy with preeclampsia to correlate clinical & pathological changes with radiological findings. **Materials and Methods:** One hundred pregnant cases, aged 18-45 years, were included in the study. Patients were allocated into 2 groups each and were followed in the study for total ante-natal duration. Group 1 (n=50) was the Control group, which included mothers with normal pregnancy. Group 2 (n=50) included mothers with preeclampsia and eclampsia. The study was conducted in Department of obstetrics and Gynecology of SSH, BHU with Department of pathology, Department of Radiodiagnosis and Department of Genetic disorders, Institute of Medical Sciences, Banaras Hindu University, Varanasi between October 2018- June 2020. **Results:** Our study revealed that the mean BMI was higher in mothers with pre eclampsia than the normotensive mothers. The mean abdominal Girth of preeclampsia and eclampsia patients were significantly lower than the control as most of cases were IUGR or preterm in the cases. Out of 50 cases in study group, in 36 patients (72%) histopathology of placenta shows pathological changes. Preeclampsia is associated with a higher prevalence of vascular and villous changes in placenta. Our study revealed that in 20 patients (40%) cerebroplacental ratio is abnormal. 34 patients (68%) shows high pulsatility index in uterine artery Doppler study. 22%of neonates are with Apgar score. **Conclusion:** Our study revealed that Preeclampsia was observed to be associated with a higher prevalence of vascular and villous changes in placenta, impaired placental circulation leads to decrease growth of placenta and fetus. The mRNA expression of VEGF gene was significantly higher in preeclampsia women.

KEYWORDS :

INTRODUCTION:

Pre-eclampsia (PE) is a pregnancy specific syndrome that can affect virtually every organ system. It affects 5-10% of pregnancies worldwide and 4.6% of pregnancies in India. The prevalence of pregnancy with hypertension was reported to be 8.3% (n=11266) with the prevalence of preeclampsia being 11.71% with a total of 15784 cases. Poor placentation is associated with an imbalance of circulating vasoactive factors and, in turn, leads to maternal vascular maladaptation with associated systemic endothelial dysfunction. Maternal organ systems that are susceptible to excessive inflammation and endothelial damage are the CNS, lungs, liver, kidneys, systemic vasculature, coagulation, and the heart—the placenta and fetus are also at risk. HELLP syndrome complicates 10–20% of cases of severe pre-eclampsia, and develops mostly preterm (50%). In 20% of women, however, it presents in late gestation, or in 30% post-partum. Eclampsia, complicating 1–2% of severe preeclampsia, 16 is defined as the occurrence of tonic-clonic seizures in a pregnant or recently delivered woman that cannot be attributed to other causes.

MATERIALS AND METHODS:

The study was conducted in Department of obstetrics and Gynecology of SSH, BHU with Department of pathology, Department of Radiodiagnosis and Department of Genetic disorders, Institute of Medical Sciences, Banaras Hindu University, Varanasi between October 2018- June 2020. One hundred pregnant cases, aged 18-45 years, were included in the study. Patients were allocated into 2 groups each and were followed in the study for total ante-natal duration. Group 1 (n=50) was the Control group, which included mothers with normal pregnancy. Uncontrolled diabetes and hypertension were excluded from the control group. Group 2 (n=50) included mothers with preeclampsia and eclampsia. Patients with age <18 years and > 45 Years, those with presence of HIV/ AIDS, HBsAg, HCV and those with known medical disorders like malignancy or significant comorbidity, patients with chronic renal disease and heart disease were excluded from study.

The clinical features and detail history of each case were recorded systematically. History included age, parity, socioeconomic status, previous history of PIH, family history of PIH and gestation age at enrollments. In the general examination built, pallor, icterus, pulse, B.P., pedal edema, and body mass index were noted. Systemic examination included the CNS, respiratory and the CVS system. Per abdominal examination was carried out to see the presentation, symphysio fundal height (in cm), abdominal girth (In inches), liquor volume and fetal heart rate. Blood sample from cases and controls were collected in plain vial and EDTA coated vials for antenatal investigations includes ABORH, CBC, LFT, RFT, uric acid, Blood sugar(fasting and post prandial), urine routine and microscopy, HIV, HBsAg, HCV and VDRL. Dimension of placenta was measured (in cm) and weighed (in gm) after delivery and evaluated macroscopically for pathological changes. Biopsy was taken and examined microscopically after fixing in formalin and stained with hemoatoxylin and eosin.Placental tissue collected in normal saline and cord blood in serum vial. Color Doppler was perfomed using pulsed wave duplex color Doppler ultrasonography.

RNA isolation

Isolate the RNA from placental tissue by using TRIZOL reagent with standard manufactured protocol. In brief, we take 50 to 100 mg tissue in 1 ml and homogenize with the glass-Teflon or power homogenizer in 1.5 ml tube. After that, add 0.2 ml of chloroform and vortex samples vigorously for 15 seconds

and incubate at room temperature for 3 minutes. Centrifuge the samples at 12000-x g for 15 minutes at 40C. Following centrifugation, the mixture separates into lower red, phenol chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Transfer upper aqueous phase carefully without disturbing the interphase into fresh tube. After separating aqueous add and mix with 0.5 ml of isopropyl alcohol, incubate at room tamp for 10 minutes. After incubation, Centrifuge the samples at 12000-x g for 15 minutes at 4 0C. Remove the supernatant and wash the RNA pellet once with 1 ml 75% ethanol. Mix the samples by vortexing and Centrifuge the samples at 7,500-x g for 15 minutes at 40C. Repeat above washing procedure once. Remove all leftover ethanol. Air-dry or vacuum dry RNA pellet for 5-10 minutes. Finally resuspend the RNA pellet into 50 μl of DNase RNase free water. Amount and purity of RNA will be determined by using Nanodrop (ND1000) and spectrophotometer. Store the sample at -800C. Complementary DNA (cDNA) synthesis Before cDNA synthesis all RNA samples were treated with DNase1 (Thermo Scientific) to remove any traces of genomic DNA. This was done according to the manufacturer's instruction with one exception; due to low RNA content in the samples 0.1 µl of DNase Inactivation Reagent were added for each µl of total volume. After DNase1 treatment prepare 20 µl mix reaction which containing 1µg total RNA, random primer and 10 mM dNTP (Invitrogen). This mixture was incubated for 5 min at 65°C and then cooled on ice for 1 min. At this point the volume of the mix was increased to 20 µl by adding 5x Superscript First strand buffer, 200 U of Superscript RT III and DTT (all from Invitrogen). After a 5 min incubation at 25°C the mixture was incubated for 60 min at 50°C, followed by 15 min incubation at 70°C. In the end the cDNA samples were diluted 10 x by adding 180 µl of MQ-water.

Real-time PCR (quantitative qPCR)

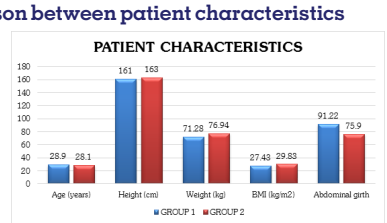
The quantitative real-time PCR was performed to evaluate the mRNA expression level of VEGF gene in placental tissue. In brief, we isolate RNA from whole tissue through trizol reagent using a standardized protocol.. cDNA synthesis were carried out with 1 µg of RNA using High Capacity cDNA Reverse Transcription Kit. qRT-PCR will be performed using ABI prism 7900HT system (Applied Biosystems, USA). For real time assay reactions 2× Power SYBR Green PCR master mixes will be used (as per provided by the manufacturer). Each PCR reaction were carried out in triplicates for each sample. Dissociation reaction were also carried out for each primer to check the specificity of primers. The comparative Ct method for relative quantification (ΔΔCt method), which describes the change in expression of the target gene in a test sample relative to a calibrator sample, were used to analyze the data. Data were analyzed using 7900HT Sequence Detector System software version 2.2.1 (Applied Biosystems, USA). Results will be expressed relative to the housekeeping gene (GAPDH).

List of primers

Gene	Forward Primer	Reverse Primer
GAPDH	GGGCATCCTGGGCTACACTGA	TCCACCACCCTGTTGCTGTAG
VEGF-A	CAGAGGAGGAGGGCAGAAT	ATCGCATCAGGGGCACACAG

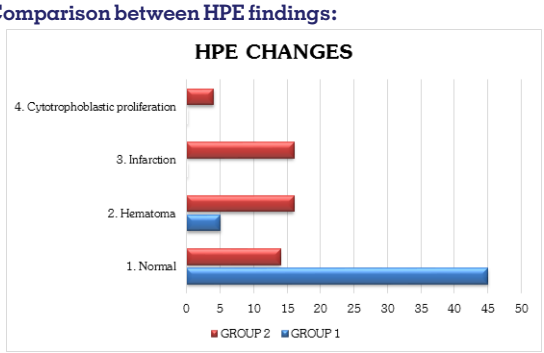
RESULTS:

Comparison between patient characteristics



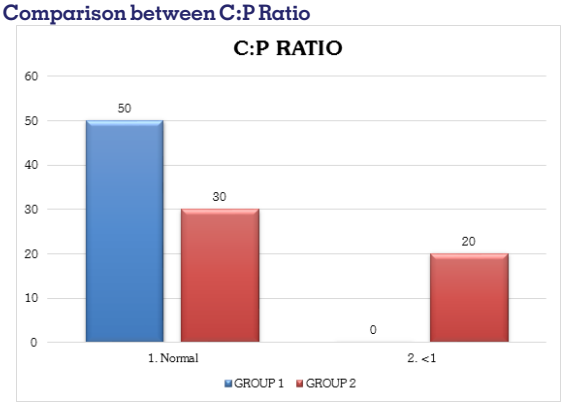
Women in both groups, were similar with regards to age, height, weight and BMI. The mean abdominal Girth of in pre-eclamptic patients was significantly lower than the control as most cases were IUGR or preterm. The age of the patients in the case of PIH ranges from 20- 40 years with the mean age (28.10+5.54) maximum patients of PIH were in age group of 25-30 years.

Comparison between HPE findings:



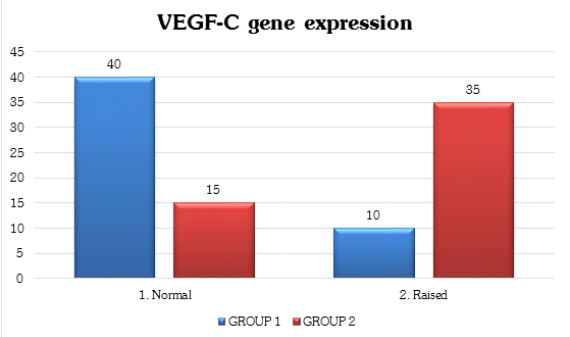
Among pre-eclampsia cases, in 36 patients (72%) histopathology of placenta shows pathological changes. Pre-eclampsia is associated with a higher prevalence of vascular and villous changes in placenta.

Comparison between C:P Ratio

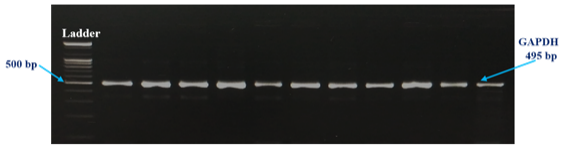


Out of 50 pre-eclampsia and eclampsia cases, cerebro-placental ratio was abnormal in 20 patients (40%). It reflects increase in placental resistance and reduction in the fetal brain vascular resistance.

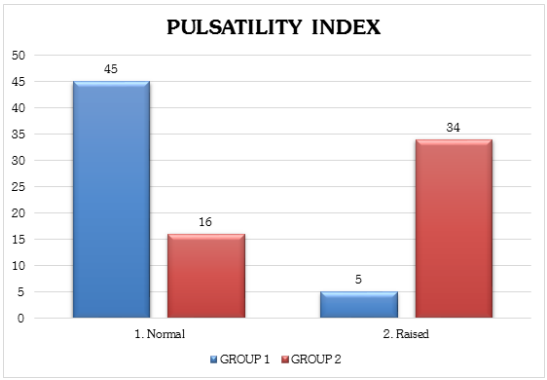
Comparison between VEGF-C gene expression



The mRNA expression of VEGF gene was significantly higher in preeclampsia women.

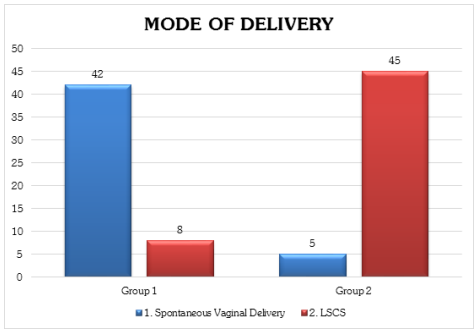


Comparison between Pulsatility Index of uterine artery



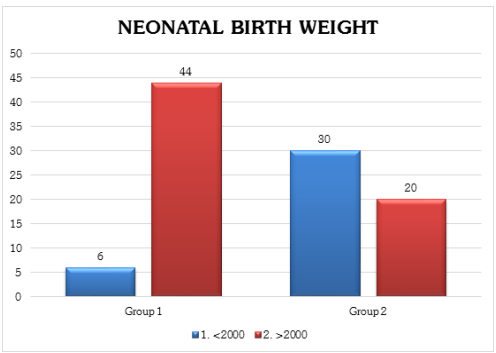
Significantly high pulsatility index in uterine artery Doppler study was observed in 34 pre-eclampsia patients (68%) as compared to control group.

Comparison of Mode of delivery:



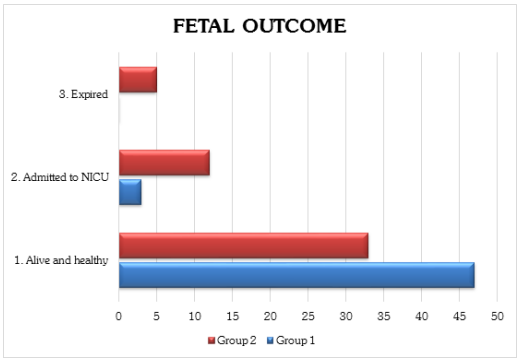
Out of total 50 preeclampsia and eclampsia patients, 90% underwent LSCS and 10% underwent SVD which was significantly high as compared to 16% LSCS and 84% SVD in the control group.

Comparison of Neonatal Birth Weight



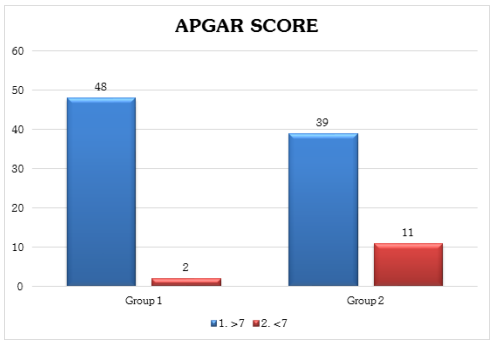
In the control group 12% babies were LBW which was lower as compared to 60% LBW babies in the pre-eclampsia and eclampsia cases, which was highly significant.

Comparison of fetal outcome



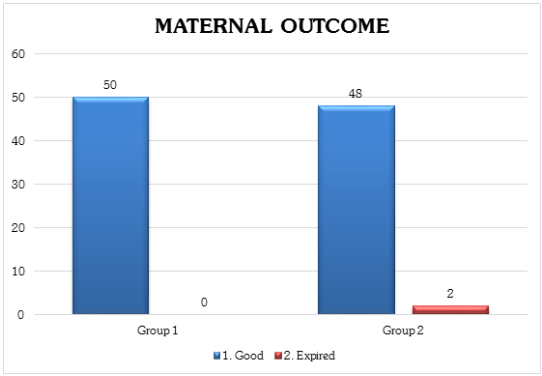
Among pre-eclampsia and eclampsia cases, in 66% babies were born alive and healthy, 24% required NICU support and 10% expired at or after birth which was significantly higher than the control group in which 94% of babies were alive and healthy, 6% required NICU support and none of the babies expired.

Comparison of APGAR at 1 & 5 min score



The APGAR score at 1 & 5 min were comparable for both groups with no statistical significance.

Comparison of Maternal Outcome



None of the mothers expired in the control group whereas only 4% maternal mortality was observed in pre-eclampsia and eclampsia cases, which was statistically not significant.

DISCUSSION:

Pregnancies complicated by pre-eclampsia show an increase in maternal and perinatal morbidity and mortality. Pre-eclampsia, a life threatening complication of pregnancy, is characterized by the onset of high blood pressure and proteinuria. The presentation of pre-eclampsia is highly variable, but generally includes the combination of maternal hypertension and proteinuria. The etiology of the disease is likely multifactorial.

The present study was conducted to study the clinical picture, Radiological pathological and Genetic factor in Pregnancy with pre-eclampsia and eclampsia and to correlate clinical & pathological changes with radiological findings.

Our study revealed that the mean BMI was higher in mothers with pre eclampsia than the normotensive mothers.

Our study revealed that the mean abdominal Girth of preeclampsia and eclampsia patients were significantly lower than the control as most of cases were IUGR or preterm in the cases.

The root cause of preeclampsia is the placenta. Preeclampsia begins to abate with the delivery of the placenta and can occur in the absence of a fetus but with the presence of trophoblast tissue with hydatidiform mole.

We observed that out of 50 cases in study group, in 36 patients (72%) histopathology of placenta shows pathological changes. Preeclampsia is associated with a higher prevalence of vascular and villous changes in placenta.

In our study, we observed that in preeclampsia, impaired placental circulation leads to decrease growth of placenta and fetus. 2 Gross pathological changes are most common with severe preeclampsia occurring preterm. The characteristic placental changes of preeclampsia would be predicted to be those associated with placental ischemia.

Our study revealed that in 20 patients (40%) cerebroplacental ratio is abnormal. It reflect increase in placental resistance and reduction in the fetal brain vascular resistance. CPR reflects fetal adaptation to placental chronic hypoxia and appears to be more sensitive than the Doppler ultrasound of the umbilical and middle cerebral artery by detecting clinically unrecognized fetal compromise.

In our study we also determined the VEGF levels in patients with preclamsia and eclamsia. We found that the mRNA expression of VEGF gene was significantly higher in preeclampsia women.

In our study, 34 patients (68%) shows high pulsatility index in uterine artery Doppler study.

In our study, 22%of neonates are with Apgar score <7 in preeclampsia and eclampsia. Main factor determining perinatal mortality was lack of regular antenatal checkups, complicated cases of preeclampsia and eclampsia and lack of awareness regarding significance of symptoms like decrease fetal movement and late arrival at hospital.

In our study, there is significant decrease in birth weight among neonates born to mother with preeclampsia as compare with those born to normotensive mother. Preeclampsia is independently associated with development of IUGR.

CONCLUSION:

Our study revealed that various clinical and pathological changes with radiological findings in preeclampsia patients.

The mean BMI was higher in mothers with pre-eclampsia than the normotensive mothers.

The mean abdominal Girth of preeclampsia and eclampsia patients were significantly lower than the control as most of cases were IUGR or preterm in the cases.

Preeclampsia was observed to be associated with a higher prevalence of vascular and villous changes in placenta. The placenta plays a central role in the origin and pathophysiology of preeclampsia.

Here, it was concluded that a cardinal sign of placental hypoxia, that is, infarction, correlates to the clinical severity of the disease and fetal outcome.

We observed that in preeclampsia, impaired placental circulation leads to decrease growth of placenta and fetus.

Cerebroplacental ratio was found to be abnormal in patients with preclamsia.

The mRNA expression of VEGF gene was significantly higher in preeclampsia women.

Out of 50 cases, 60% of the patient delivered babies who had birth weight.

Out of 50 cases, 66% gave birth to alive and healthy babies while 5%babies expired due to fetal distress.

78% babies delivered to preeclamptic patient had good apgar score at 1 & 5 min. of life. 22% had low apgar score.

Out of 50 cases, 2 patient (4%) expired and 96% had good outcome.

Thus the present study revealed various clinical and pathological changes with radiological findings in preeclampsia patients.

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