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Immunohistochemical Expression E-Cadherin in the Basement Membrane of Cytotrophoblast, Syncytiotrophoblast and Blood Vessels of Placenta of Pre-Eclampsia

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Abstract: ***Introduction**: Pre - eclampsia is a severe pregnancy complication characterized by elevated blood pressure and proteinuria occurring after 20 weeks of gestation. It affects 2 - 5% of pregnancies worldwide. During early pregnancy, there is a temporary decrease in E - cadherin expression in trophoblastic cells as they invade the placenta. However, the role of E - cadherin in regulating placental vascularity remains poorly understood. This study aims to analyse the intensity and distribution of E - cadherin staining in the trophoblastic villi of healthy and pre - eclampsia placenta patients. **Materials & Methods**: This study was conducted in the Department of Pathology from September 2022 to December 2023. A total of 160 placenta samples were examined, consisting of 80 pre - eclampsia cases and 80 healthy controls. Immunohistochemistry was performed using antibodies against E - cadherin to evaluate its expression. The expression of E - cadherin was compared between cases and controls and correlated with clinicopathological parameters. Statistical analysis was conducted using the Chi - square test, and the mean and standard deviation were calculated. A p - value of <0.05 was considered statistically significant. **Results**: The average age of participants was twenty - six years. Gross and microscopic findings such as necrosis, calcification, and thrombosis were more prevalent in pre - eclampsia placentas than in healthy controls, and these differences were statistically significant. Immunohistochemical analysis showed high (grade 3) and continuous E - cadherin expression in the majority of healthy placental villi. In contrast, pre - eclampsia placentas exhibited varied E - cadherin expression (grades 0, 1, 2, & 3) and discontinuity in staining, which were statistically significant findings. **Conclusion**: This study demonstrates that E - cadherin expression, as evaluated by immunohistochemistry, is reduced in intensity and discontinuous in the cytotrophoblast, syncytiotrophoblast, and blood vessels of pre - eclampsia placentas compared to normal placentas. These alterations may reflect abnormalities in maternal fetal junction, contributing to the development of pre - eclampsia.*

Keywords: Pre - eclampsia placenta, E - cadherin, Immunohistochemistry

1. Introduction

Pre - eclampsia, is a complication during pregnancy which is characterized by increased blood pressure and proteinuria after 20 weeks, affecting 2 - 5% of all the pregnancies globally and carries significant risks for both mother and baby.1 While the exact pathogenesis remains unclear, growing evidence suggests an imbalance in factors regulating blood vessel growth, transport across the vasculosyncytial membrane from mother to the fetus may be compromised might contribute to the development of pre - eclampsia.2 E - cadherin is a protein linking structure that associates cells together which is crucial for early development, cell to cell interaction and its expression is known to be aberrant in various cancers. During pregnancy in the initial months there is a temporary decrease in E - cadherin expression within trophoblastic cells as they invade placenta, nevertheless the function of E - cadherin in regulation of placental vascularity is not fully understood.

Aims & Objectives

1) To determine the Immunohistochemistry (IHC) expression of E - cadherin in the placenta of healthy normal pregnancy and the placenta of pre - eclampsia.
2) To study the correlation of intensity of expression of E - cadherin and the severity of pre - eclampsia.

2. Materials & Methods

Study Design: Cross - sectional observational study.

Source of Data: All the placenta specimens which were sent to the Department of Pathology were examined thoroughly and the details of the procedure was explained to the patient in their own language and consent was taken.

Study Duration: September 2022 – December 2023

Sample Size:

$$n = \frac{Z^2_{1-\propto} * p * q}{d^2}$$

- Z = Standard normal variant (1.96)
- p = prevalence = 8%
- q = 1 – prevalence
- d = absolute error (6%)
- Therefore n = 79.65

Sample size is estimated based on 8% prevalence of pre - eclampsia.3 Considering an absolute error of 6% with 95% confidence interval the estimated minimum sample size for each group of the present study was 80 and the total sample size was **160**.

Collection of Data:
A total of 80 placentas with > 28 weeks of gestational age with pre - eclampsia was included in the study and 80 placentas from normal antenatal cases attending at RLJH & Research Centre in Sri Devaraj Urs Medical College, Kolar from 2022 – 2024 was included in this study.

Inclusion Criteria:
All patients diagnosed with pre - eclampsia > 28 weeks of gestation age complying with criteria of pre - eclampsia who underwent delivery (normal and caesarean section) at R L Jalappa Hospital & Research Centre between 2022 - 2024.

Exclusion Criteria:
1) Chronic hypertension
2) Congenital abnormality in new born
3) Twin pregnancy
4) Hypothyroid patient
5) Clinically detected other medical conditions like Heart disease, Systemic lupus erythematous, Rh incompatibility.

Methodology

Method Of Collection:
1) The placenta was collected immediately after delivery from mild, moderate, severe pre - eclampsia cases and healthy groups and is washed in tap water to eliminate any blood clots.
2) Gross inspection was done noting weight, diameter, thickness, no. of cotyledons on the maternal surface, calcification, infarction and umbilical cord vessels and knots.
3) The placenta is sliced at regular intervals of 0.5cm and gross abnormalities were detected (Bread and slice method)
4) The whole specimen was left for fixation in 4% formalin for more than 48 hrs.

Sampling Procedure:
1) Five sections were taken from each central and peripheral areas.
2) Additional sections were taken from abnormal areas.
3) Tissue sections 5 micrometre thickness were cut from paraffin embedded blocks and stained by conventional H&E stain.
4) Placental changes were compared with the severity of the pre - eclampsia (mild, moderate and severe) with that of control group.

MMUNOHISTOCHEMICAL STAINING:

Protocol:
IHC staining was performed on 10% formalin - fixed (fixed for >48hrs at 25 degree Celsius) paraffin - embedded 4 micro meter tissue sections which were taken on coated slides. IHC was performed using peroxidase anti - peroxidase method. The details of the primary antibody are as follows -

Table 2: Antibody, clone, species used in IHC staining -

Antibody	Clone	Species	Producer	Control	Stain
E - Cadherin	EP6 Monoclonal	Rabbit	Pathn Situ Biotechnologies	Colon Cancer	Membrane

Positive and negative controls were run simultaneously for all cases.

Table 3: After the process of IHC the slides were interpreted and documented as below -

Basement Membrane of	Grading			
Cytotrophoblast	-	+	++	+++
Syncytiotrophoblast	-	+	++	+++
Blood vessels	-	+	++	+++

The expression was graded as –
- (0) = No staining
- + (1) = Mild discontinuity in the staining
- ++ (2) = Moderate discontinuity in the staining
- +++ (3) = Strong continuity in the staining

Statistical Analysis
All the data collected was entered into Microsoft excel data sheet and was analyzed by SPSS 24 (Statistical Package for Software Sciences 24) version software in India. Quantitative data was represented using mean and standard deviation. Independent t test was performed as test of significance to see the mean difference. All the Qualitative data was demonstrated in the form of frequencies and proportions. Chi square test was used to look for difference between the groups. P value <0.05 at 95% CI was considered statistically significant.

3. Results

A total of 160 placentas were included out of which 80 (50%) placentas were from normal term pregnancy which formed healthy control group and 80 (50%) placentas were from pregnancy with pre - eclampsia (BP>140/80). Of these 20 cases were of mild preeclampsia, 20 cases of moderate preeclampsia and 40 cases were of severe pre - eclampsia. All the mothers in the control group and the pre - eclampsia group fulfilled the selection criteria.

Grossly areas of calcification, fibrosis, necrosis and thrombosis were identified. Microscopically on H&E staining, calcification appeared as areas of dense, thick dark blue staining. Fibrosis is a result from the formation of excess fibrous connective tissue due to chronic inflammation or injury and this is seen as regions of increased collagen deposition. Necrosis is identified as acellular, eosinophilic areas with a loss of tissue architecture. Excessive syncytial knots, clusters of syncytial cells, are indicative of placental hypoxia. Peri - villous edema is seen as widened spaces between the villi, caused due to fluid accumulation were seen.

Table 1: Expression of E - cadherin among cases and control groups

Expression of E - cadherin	Control (n=80)	Pre - eclampsia (n=80)
Present	70 (88%)	58 (73%)
Absent	10 (12%)	22 (27%)

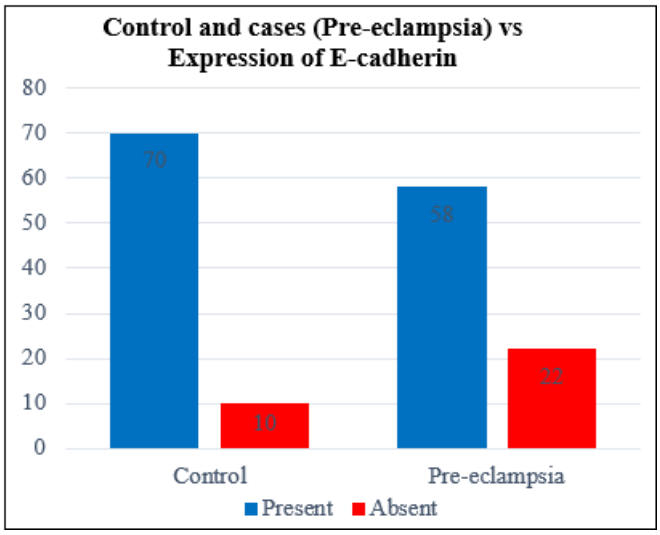

Chart 1: Expression of E - cadherin among healthy control groups and cases

The table compares the presence and absence of E - cadherin expression between control and pre - eclampsia groups.

Higher Presence of E - cadherin in Healthy Group: In the healthy group majority of cases (88%) showed presence of E - cadherin with continuous staining among the placental villi and only a small percentage (12%) showing its absence.

Lower Presence of E - cadherin in Preeclampsia Group: In the preeclampsia group less number of cases showed the presence of E - cadherin with discontinuous staining compared to the healthy group. Absence of E - cadherin was more in the pre - eclampsia group than in the control group.

This indicates that E - cadherin expression is significantly decreased in preeclampsia cases compared to the healthy group and indicates that preeclampsia is linked with decreased E - cadherin expression.

The chi square data analysis is done for the presence of expression of E - Cadherin in normal placenta (healthy groups) and the pre - eclampsia placentas.

Overall, it showed a significant association between them with the p value of 0.017706 ($p < 0.05$) which is statistically significant.

Table 2: Severity of expression of E - cadherin among cases and healthy control groups

Expression of E - cadherin	Control (n=80)	Pre - eclampsia (n=80)	Pre - eclampsia		
			Mild (n=20)	Moderate (n=20)	Severe (n=40)
No	10 (12%)	22 (28%)	2 (10%)	5 (25%)	15 (38%)
Low	15 (19%)	20 (24%)	5 (25%)	5 (25%)	10 (25%)
Intermediate	20 (25%)	26 (32%)	8 (40%)	8 (40%)	10 (25%)
High	35 (44%)	12 (6%)	5 (25%)	2 (10%)	5 (12%)

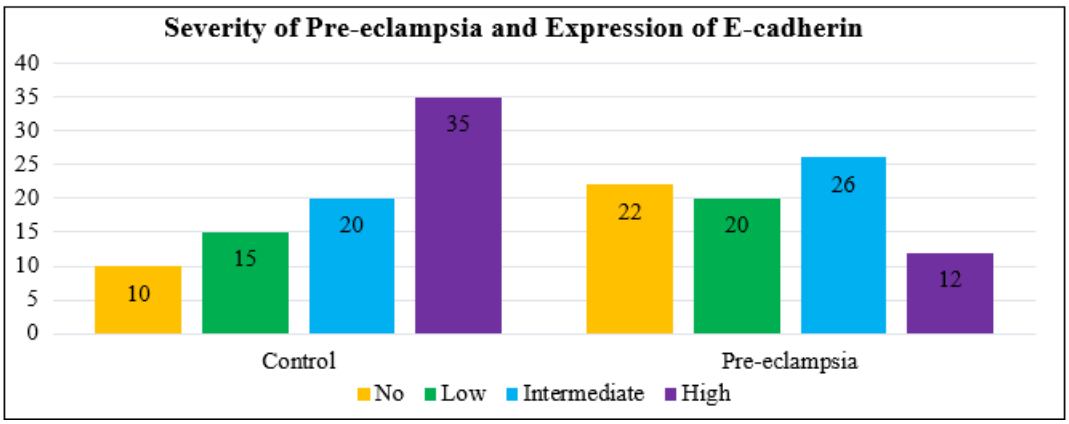

Chart 2: Severity of expression of E - cadherin among control groups and cases

The intensity of expression of E - cadherin was measured in control group and pre - eclampsia.

The intensity of expression grading was done according to the IHC scoring expression as mentioned above.

When the intensity of expression of E - cadherin was seen in the control group, predominant cases that is (44%) showed high expression which is relating to grade 3 (Fig 4) of intensity of expression of E - cadherin. Similarly (25%) of the cases showed intermediate expression with which is corresponding to grade 2 (Fig 3) of intensity of expression of E - cadherin. Finally, the remaining (19%) and (12%) of the cases showed low and no expression which is corresponding to grade 1 (Fig 2) and 0 (Fig 1) respectively of intensity of expression of E - cadherin.

However, in overall preeclampsia, a higher percentage of cases (28%) showed no E - cadherin expression which is correlating to grade 0 (Fig 1) of intensity of expression of E - cadherin, when compared to the control group. Only a small percentage (6%) showed high expression which is corresponding to grade 3 (Fig 4) of intensity of expression of E - cadherin.

In Mild Pre - eclampsia majority of cases (65%) showed intermediate or high expression which is grade 2 and 3 (Fig 3 and 4 respectively).

In Moderate Pre - eclampsia the intermediate expression that is grade 2 (Fig 3) was most common (40%), but there was a significant proportion with no (25%) or low (25%) expression which is grade 0 and 1 (Fig 1 and 2) respectively.

In Severe Pre - eclampsia majority of cases (63%) showed no or low expression which is grade 0 and 1 (Fig 1 and 2) respectively and only 12% showing high expression which is correlating to grade 3 (Fig 4) of intensity of expression of E - cadherin.

The chi square data analysis is done for the level expression of E - cadherin and the severity of pre - eclampsia showed a significant association between them with the p value of 0.003108 ($p < 0.05$) which was statistically significant.

Therefore, this suggests that E - cadherin expression tends to decrease with increasing severity of pre - eclampsia and the most severe cases have the lowest levels of E - cadherin expression.

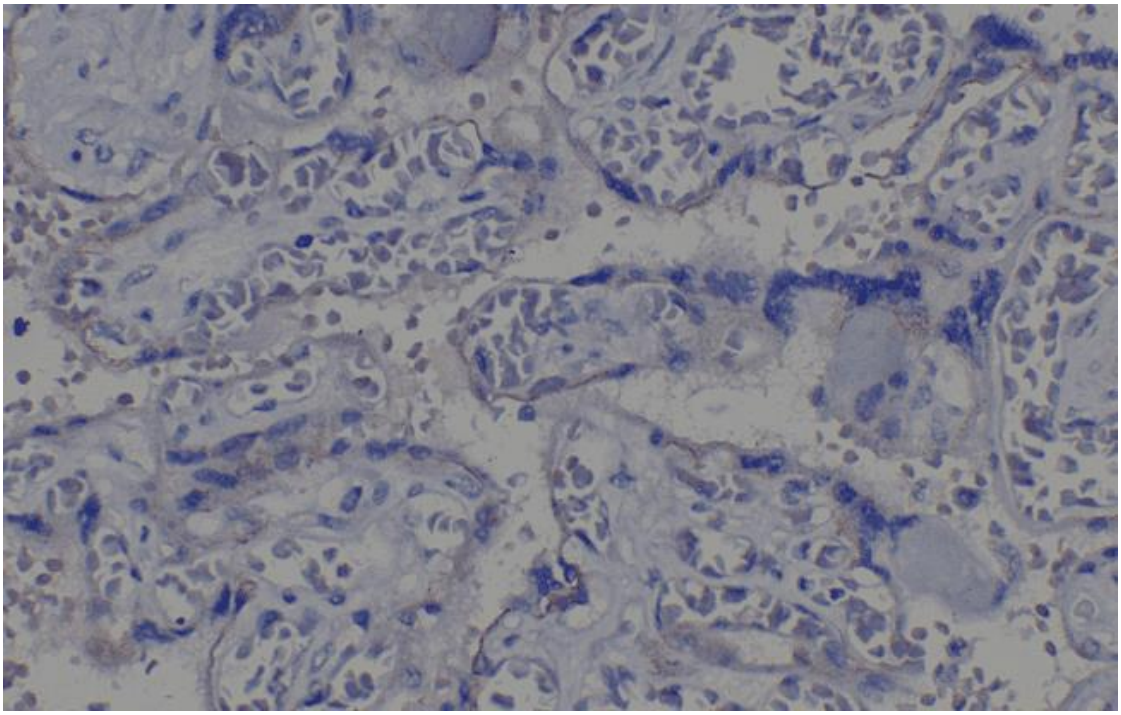

Figure 1: Shows no IHC expression which is grade 0 of intensity of expression of E - cadherin in pre - eclampsia placenta in 100x magnification.

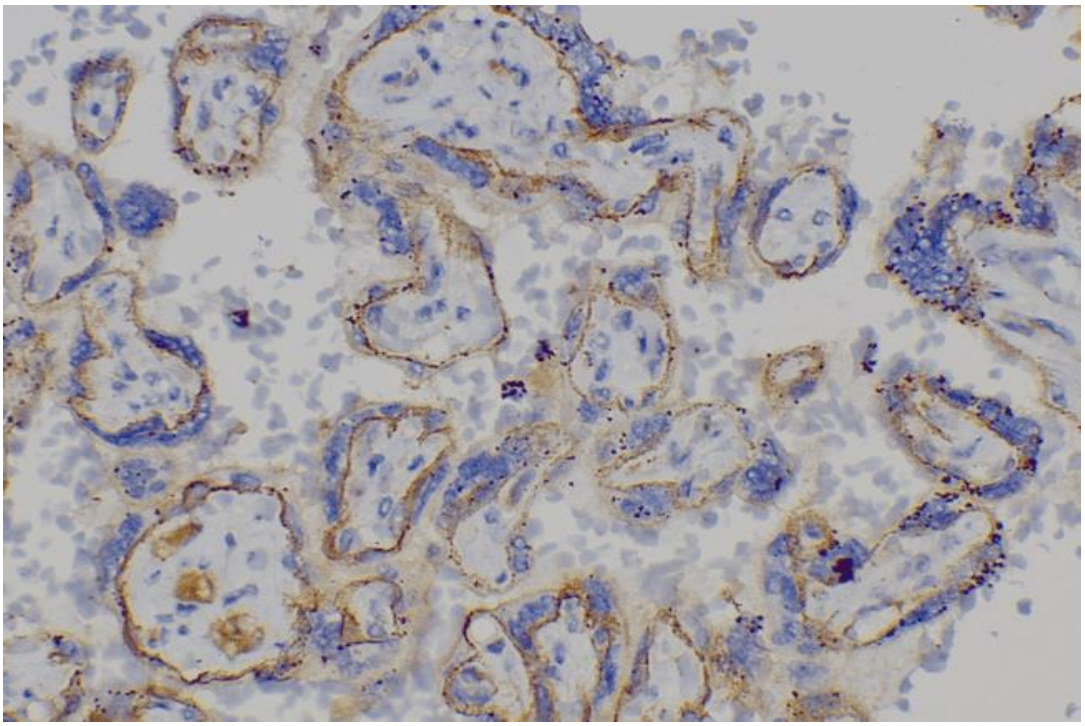

Figure 2: Shows the mild IHC expression which is grade 1 of intensity of expression of E - cadherin in pre - eclampsia placenta in 100x magnification.

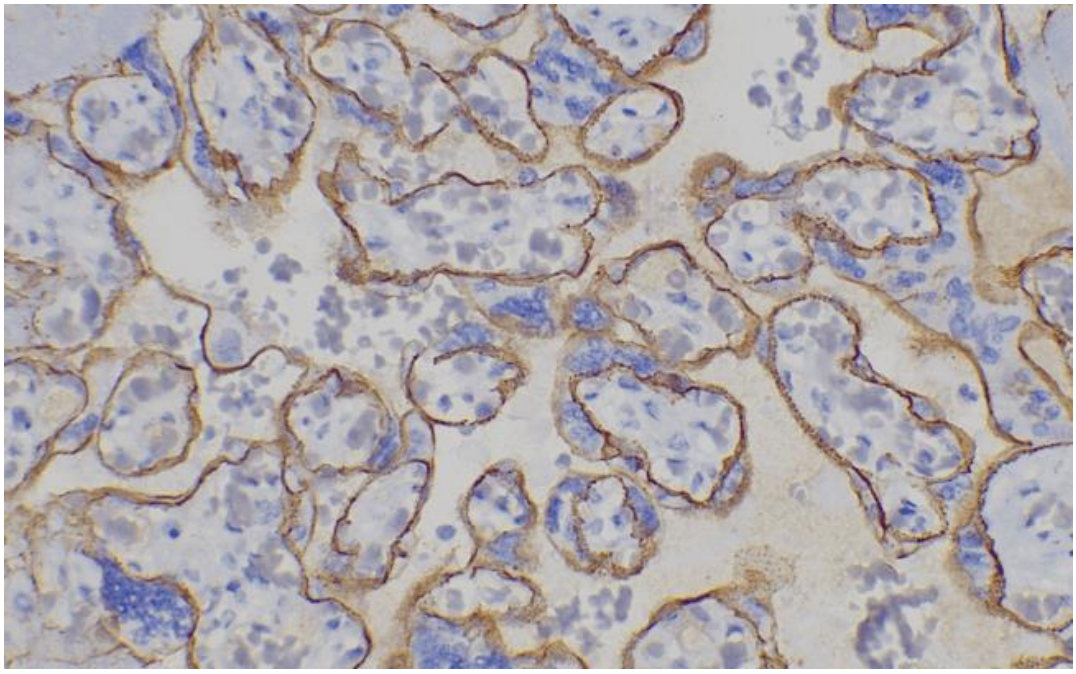

Figure 3: Shows the moderate IHC expression which is grade 2 of intensity of expression of E - cadherin in pre - eclampsia placenta in 100x magnification.

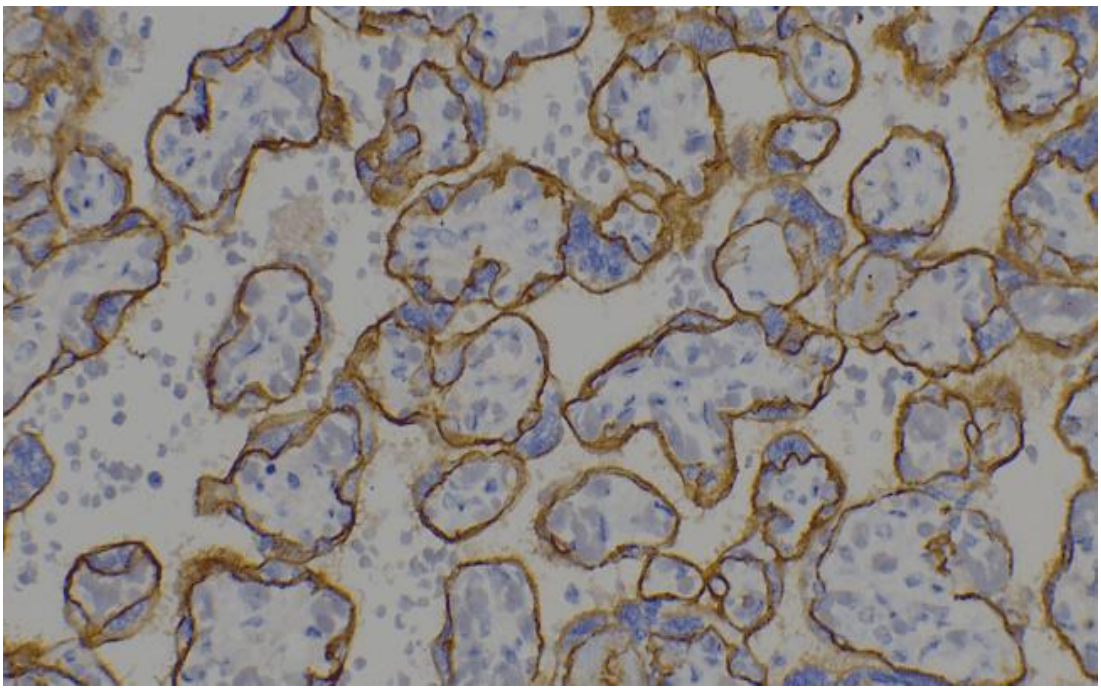

Figure 4: Shows strong IHC expression which is grade 3 of intensity of expression of E - cadherin in placenta in 100x magnification.

4. Discussion

The placenta is a complex organ that connects mother & fetus, and its function is highly influenced by its anatomical structure. The morphology and cellular architecture of the placenta are crucial for adequate oxygen transport from the mother to the baby. Successful placental development is essential for fetal growth and well - being greater than 20 weeks of gestation and is necessary for adequate maternal blood supply to the placenta.4 The placental architecture is altered in diseases like pre - eclampsia, gestational hypertension, gestational diabetes mellitus that affects the mother and the fetus as well.

As age advances, women experience significant physiological changes that can impact vascular health that is increasing the likelihood of developing pre - existing conditions such as chronic hypertension, diabetes, and renal disease. These conditions are well known risk factors for pre - eclampsia. Additionally, older women tend to have higher baseline levels of inflammatory markers, which contribute to an enhanced inflammatory response. This heightened inflammation plays a critical role in the pathogenesis of pre - eclampsia, further elevating the risk of developing this condition.

In the current study the expression of e - cadherin was assessed in the normal placenta and the pre - eclampsia

placenta. E - cadherin in healthy placentas was more positively expressed that is nearly 88% showed the presence of e - cadherin, a protein that is involved in cell - to - cell adhesion and only a small portion (12%) lacked it. E - cadherin expression is less common in pre - eclampsia placentas when compared to healthy placentas that is almost 73% cases and the absence of e - cadherin expression was seen in 27% of the cases in this group. Therefore, pre - eclampsia likely reduces E - cadherin expression and this difference suggests that pre - eclampsia is associated with a decrease in E - cadherin levels in the placenta. A statistical analysis (chi - square test) confirmed a significant association (p - value = 0.017706, less than 0.05) between E - cadherin expression in the normal and pre - eclampsia placenta.

During pregnancy, E - cadherin, a cell - to - cell adhesion molecule is important for maintaining epithelial integrity and plays a crucial role in the development and functioning of the placenta. E - cadherin expression in trophoblastic cells temporarily decreases during the process of placental invasion in the first and second trimesters. This temporary decrease of E - cadherin enables the invasive capability of trophoblasts, allowing them to permeate and remodel the maternal uterine tissue effectively.5

However, as the pregnancy progress E - cadherin is expressed strongly and circumferentially in the placental villi, which is essential for maintaining the structural and functional integrity of the placental barrier and helps in completion of the trophoblast invasion and the establishment of the placental villous architecture, which in turn helps in nutrient and gas exchange between the mother and the fetus.

Shallow trophoblast invasion is due to downregulation of E - cadherin and other invasion - related mechanisms, which is a hallmark for pregnancy - induced hypertension (PIH) and pre - eclampsia (PE). Here, the spiral arteries stay narrow and high - resistance causing reduced placental perfusion. This inadequate blood flow leads to placental hypoxia, oxidative stress, and the release of antiangiogenic factors into the maternal circulation, contributing to the systemic manifestations of PE, such as hypertension and proteinuria.6

In a recent study E - cadherin expression was significantly higher in the control group (normal placentas) and was less or weakly expressed in the pre - eclampsia placentas, these findings are similar to those of the present study.2 Another study stated that expression of e - cadherin was higher in pre - eclampsia cases when related to the normal control cases which was not similar with present study. Similarly, another study stated that e - cadherin expression by western blot technique was not detectable in normal placentas but was highly expressed in preeclamptic placentas which was not correlating with the present study.

In present study, intensity of expression e - cadherin was evaluated in both control and pre - eclamptic groups. The expression levels were graded from no expression to high expression that is grade 0 to 3.

In the control group, major cases exhibited high E - cadherin expression that is grade 3. On the contrary in pre - eclampsia group a higher number of cases that is 28% showed no E - cadherin expression (grade 0) compared to control group.

Among the mild pre - eclampsia cases, a higher number of cases that is 65% showed intermediate and high expression (grade 2 and 3). In moderate pre - eclampsia cases, intermediate expression that is grade 2 was most common. For severe pre - eclampsia, highest number of cases that is 63% showed no and low expression that is grade 0 and 1.

Chi - square data analysis showed positive correlation between that level of E - cadherin expression and severity of pre - eclampsia with a p - value of 0.003108 ($p<0.05$) which is showing statistical significance.

A recent study showed detailed examination of E - cadherin staining patterns which revealed significant differences between patients with pregnancy - induced hypertension and pre - eclampsia compared to the normal pregnant control group.2 Specifically, the study found that patients with pregnancy - induced hypertension and pre - eclampsia exhibited a loss of E - cadherin staining continuity. This means that the staining of E - cadherin, a protein crucial for cell - to - cell adhesion, was disrupted in these patients.

Furthermore, the study stated that in the pre - eclampsia and PIH groups, there was a distinct discontinuity in the staining at the basement of the villi.2 The villi are important in gaseous exchange and nutrition between mother and fetus. In normal pregnancies, the basement membrane of these villi showed continuous, uninterrupted staining for E - cadherin, indicating a healthy and intact cellular architecture.

In healthy control groups, the e - cadherin expression is predominantly continuous causing the structural integrity of normal placenta and supports cell to cell adhesion leading to normal function of placenta and nutrition to fetus. In pre - eclampsia cases, especially the severe forms there is marked discontinuity in the E - cadherin expression in the villi. This loss of continuity is associated with increased vascular resistance of uterine arteries leading to disruption in cell - to - cell adhesion and impaired placental function.

However, in the pre - eclampsia and pregnancy - induced hypertension groups, this continuous staining was disrupted, suggesting a compromised structural integrity of the placental tissue. This disruption in E - cadherin staining continuity could potentially contribute to the pathological mechanisms causing underlying pre - eclampsia and pregnancy - induced hypertension, affecting the proper functioning of the placenta.

These findings from the recent study are consistent with the results seen in present study, which also showed a significant reduction in expression of E - cadherin and staining continuity in pre - eclampsia cases compared to normal pregnancies.2 The similarity in these findings between the two studies underscores the important role of E - cadherin disruption in the pathogenesis and progression of preeclampsia and pregnancy - induced hypertension. Additionally, the observed differences in E - cadherin staining patterns between the groups were statistically significant, further supporting the correlation between reduced expression of E - cadherin and severity of these conditions. The evaluation of e - cadherin

expression can be a potential marker to know the development of pre - eclampsia.

5. Conclusion

This study indicates that the reduced intensity and discontinuity of E - cadherin staining in pre - eclampsia cases point to a defect in placental barrier function. This defect likely contributes to the development of pre - eclampsia and associated pregnancy complications. The findings suggest that disruptions in E - cadherin expression may impair the structural integrity of the placenta, thereby playing a critical role in the pathogenesis of pre - eclampsia. However, in pre - eclampsia cases with positive e - cadherin other mechanisms causing disruption of vasculosyncytial membrane needs to be investigated.

6. Limitations

We did not investigate whether similar E - cadherin alterations occur in placental disruptions associated with other gestational diseases. This means that our findings are specific to pre - eclampsia and cannot be used to other conditions affecting pregnant women. Future research should be done on larger demographic areas.

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