



## A COMPARATIVE STUDY OF THE STRUCTURAL AND HISTOPATHOLOGICAL CHANGES IN THE PLACENTAE OF HYPERTENSIVE AND NORMOTENSIVE MOTHERS AND A CORRELATION OF THESE FINDINGS WITH THE BIRTH WEIGHTS OF THEIR BABIES.

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### ABSTRACT

**Background:** The placenta being the main organ directly involved with fetal development in the womb. Being a crucial organ responsible for the pregnancy and fetal nutrition it has generated keen interest and research to understand the placental morphology. This work aims in delivering the structural changes observed in placenta of mothers with hypertension and to do a comparative analysis with respect to the new born birth weight with the normotensive mothers. **Objective:** To identify & investigate pathological changes of the placenta in cases of pregnancy-related high blood pressure & to find out correlation of such changes with neonatal birth weight as compared to that in normotensive mothers and also observation of changes in placental tissue specimens of hypertensive mothers. The study also compared fetoplacental (F-P) ratios in both groups. **Materials and Methods:** This study was done on 50 placentae. 25 mothers with normal pregnancies and 25 mothers with hypertension due to pregnancy were selected from obstetrics and gynecology patients at the Medical College, Kolkata. All samples were then examined for structural and histological changes. The neonatal birth weights were also recorded with the help of weighing scale. **Results:** In our analysis, 25 (50%) patients were hypertensive (group A) and 25 (50%) were normotensive (group B). Morphometric parameters considered in these two patient groups are placental weight, placental volume, central placental thickness, and cotyledon number. Mean differences for each of these parameters were compared and found to be statistically significant. Fetal weight was also measured in each case, the F-P ratio was observed, and the mean F-P ratio in Group A and Group B was also compared.

**KEYWORDS :** Placental morphometry, hypertension in pregnancy, histological changes of the placenta.

### INTRODUCTION

The placenta is the most critical organ which determines the fetal health in the womb and also the maternal health. The placenta helps to continue pregnancy and promotes optimum growth of the fetus. Pregnancy induced hypertension (PIH) being one most common primary cause of maternal mortality and a leading cause of fetal weakness is of utmost clinical importance.

Complications of pregnancy such as hypertension are greatly reflected in the placenta; the changes could be observed macroscopically and micro scopically. Some studies already depicted decrease in uteroplacental blood flow due to maternal vasoconstriction in PIH. This causes narrowing of major fetal trunk arteries & also causes changes in morphology of placenta in pre-eclamptic women. Fetal hypoxia can be caused by maternal vasospasm leading to distress and ultimately demise of the fetus.

The study was performed so that normal data on placental morphology, morphometry could be collected & histological changes of placenta in mothers with PIH could be observed to make a comparative analysis of the results with neonatal birth weight. Therefore, it is necessary to observe the adverse effects of hypertension on placental morphology & histology and to compare them with normal placental morphology and histology to better understand the consequences of the process of hypertension in pregnancy.

### MATERIAL AND METHODS

This study, which was comparative cross-sectional, was conducted in Medical College, Kolkata, in the Department of Anatomy, in association with the Department of Obstetrics and Gynaecology during period May 2019-May 2020.

The sample size calculation data is done using the below techniques:

- Epi Info (TM) 3.5.3 contributed to the sample size calculation.
- Microsoft excel spreadsheet is used for collecting the data

and to perform the mathematical calculations and then analysis of the same was done by using SPSS 27.0 & Graph Pad Prism V5.

- The mean and standard deviations have been calculated for numerical variables.
- The count and percentages were summarized for categorical variables.
- Unpaired proportions are compared by the Chi-square test or Fischer's exact test, as required. P-value  $\leq 0.05$  was considered to be statistically significant.

### Sample Size Justification:

So, for this study  $p=0.05$ . The method used for sample size calculation was as follows:-

$$n = \frac{4pq}{L^2} \quad [n = \text{required size of sample, } p = 0.05 \text{ (for this study), } q = 1 - p,$$

$$L = \text{Loss \% (Information loss)]}$$

### Calculation:

$$\text{Here } p = 0.05$$

$$q = 1 - p = 1 - 0.05 = 0.95$$

$$4pq = 4 \times 0.05 \times 0.95 = 0.19$$

$$L^2 = 0.0038$$

$$L = 0.0616$$

$$\text{Loss \%} = 6.16\%$$

$$n = \frac{4pq}{L^2} = \frac{0.19}{0.0038} = 50.0 = 50$$

So total of 50 patients were taken in this study. 25 Patients to be considered in the Hypertensive group & 25 Patients in the Normotensive group. So the number of patients needed for this study was obtained as 50 with a power of 89% with a 95% confidence of Interval. [1]

### Study design:

Histological and comparative cross-sectional study

### Randomization and Data Collection Forms:

- Lottery system was used for randomization in both groups.
- A series of procedure was done containing various parameters for data collection which was electronically in computer.

- The importance of the study was thoroughly explained to the husbands or legal guardians. The consent form was duly signed and then data collection was started.
- Structured interviews with a predesigned questionnaire were used for baseline information. A total of 50 full-term placentae were obtained from Medical College Kolkata's Obs & Gynaec department.
- Consent forms, case record forms and instruments such as long needles, scales, and graduated cups were the materials used to record the observations in this study. The trial was initiated only after proper ethical clearance was obtained by the institutional ethical committee.
- Out of the 50 placentae collected for this study, 25 placentae were collected from hypertensive mothers (preeclampsia/preeclampsia cases) and used as study group A. Another 25 placentae were from normotensive mothers and were used as group B because they had uncomplicated full-term deliveries.
- Control membrane-attached placentae and umbilical cords were removed approximately 30 minutes after delivery, washed with running water to remove clots, and prepared for measurement.
- After recording the measurements, the placenta was dissected for histological studies.
- All placentae harvested were then preserved in 10% formalin 30 minutes postpartum.

**Morphometric parameters measured:**

- Placental weight and birth weight were recorded by placing the placenta and neonate directly on a standardized scale for measurement [2].
- Placental volume was measured using the Archimedes principle. First, we took a beaker of water, recorded the water level and then submerged each placenta into the beaker. The water level rose due to buoyancy and a water level difference was observed. The amount of water pushed up is the volume of the placenta [2].
- Next, we measured the thickness of the central placenta, place the placenta on a flat plate and use a long needle to measure and record the thickness.
- To count cotyledons, placentas were fixed in 10% formalin for 1 hour and counted and recorded.
- Fetoplacental ratios were then calculated and recorded.
- All the data were compiled and analysed.

**Histology:**

Microscopical examination was done after tissue processing and stained with the following parameters after recording the gross characteristics of each group of placentae :-

i] Syncytial nodules - multinucleated projections of syncytial nuclei from the villous surface are observed. These are sync nodes. The number of villi with syncytial nodules was recorded and the results expressed as a percentage of the aggregate.

ii] Vascular syncytial membrane – Vascular syncytial membranes were found in villi as weakened areas of syncytiotrophoblasts that appeared to overlap and merge into the walls of adjacent dilated fetal capillaries. The number of villi with vascular syncytial membranes was expressed as a percentage of the aggregate.

iii] Fibrinoid Necrosis – Small nodules of homogeneous eosinophilic fibrinoid necrotic material was observed within the villi. In some places, this fibrinoid material may cover the entire villus. The occurrence of necrotic villi were observed & the total number of such villi was expressed as incidence.

iv] Fibrous villi (interstitial fibrosis) – Interstitial fibrosis was observed in sections and the number of fibrous villi was recorded and expressed as incidence.

The obtained numerical data were tabulated as mean, standard deviation & percentages were calculated for categorical variables. Two samples were analysed by Student's unpaired t-test. A comparative analysis of these gross and histological features of both types of the placenta (i.e., normotensive and hypertensive) was performed, and in these cases an association with reduced fetal weight, as a result, hypertensive pregnancies was observed.

**RESULTS**

In our study, 25 patients in study group A (50.0%) patients were hypertensive, and the rest 25 of the control group B were normotensive table 1.

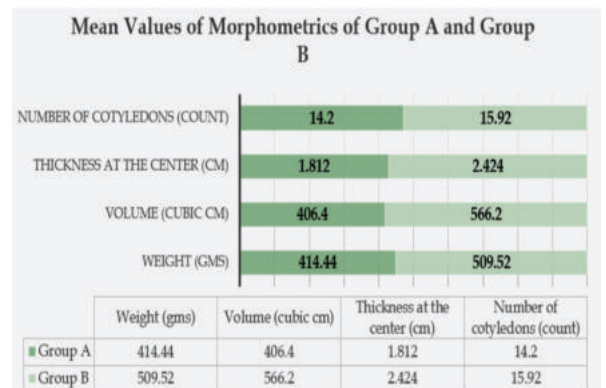
**Table 1: Distribution of Group**

Group	Frequency	Percent
A: HYPERTENSIVE	25	50.0%
B: NORMOTENSIVE	25	50.0%
Total	50	100.0%

**Table 2: Comparison between Hypertensive (Group A) and Normotensive (Group B) based on Placental Morphometry.**

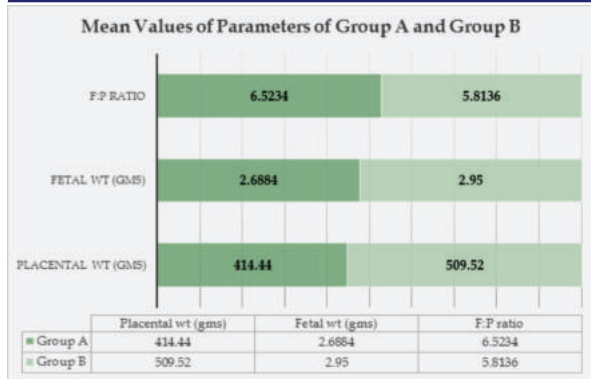
Placental parameters	GROUPS A=Hypertensive, B= Normotensive	Mean	SD	P- value obtained from t-Test.
Placental weight(gms)	A	414.4400	58.9435	<0.0001
	B	509.5200	66.6940	
Placental volume	A	406.4000	52.7478	<0.0001
	B	566.2000	80.5357	
Placental thickness at the center	A	1.8120	0.2774	<0.0001
	B	2.4240	0.3491	
Placenta number of cotyledons	A	14.2000	1.4720	0.000071
	B	15.9200	1.4697	

The morphometric data of both the groups were compared after calculation of the P-value by paired t-test and the difference of values between the hypertensive group and Normotensive Group B was observed to be statistically significant in each of the cases (p < 0.0001) as evident from Table no.2.



**Fig. 1: Graphical representation showing mean values of both the groups**

It is observed from Fig. 1, that mean Placenta wt (gms) of Group A and Group B were 414.4400 and 509.5200 respectively, the mean volume of Placenta of Group A and Group B were found to be 406.4000 & 566.2000 respectively, the mean Placenta thickness at the center (cm) of Group A and Group B were 1.8120 & 2.4240 respectively, the mean Placenta no of cotyledons of Group A and Group B were 14.2000 and 15.9200 respectively.



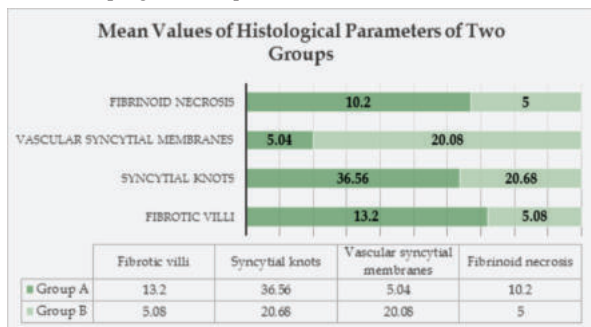
**Fig. 2: Graphical representation of Distribution of Feto-Placental ratio in both the groups**

As seen in the Fig. 2 the hypertensive Group, the mean fetal wt (kg) (mean± S.D.) of patients was 2.6884± .3246. Whereas in the normotensive Group, the mean fetal wt (kg) (mean± S.D.) of patients was 2.9500 ± .3697. The difference in mean fetal wt (kg) in both groups was statistically significant (p=0.0106).

**Table 3: Distribution of Histological parameters in Group A, Hypertensive (n=25), and Group B, Normotensive (n=25)**

Parameters	Group	Mean	SD	P-value
Fibrotic villi	A	13.2000	1.224 7	<0.0001
	B	5.0800	0.8124	
Syncytial knots	A	36.5600	4.4729	<0.0001
	B	20.6800	1.9519	
Vascular syncytial membranes	A	5.0400	1.2410	<0.0001
	B	20.0800	2.5482	
Fibrinoid necrosis	A	10.2000	1.4142	<0.0001
	B	5.0000	0.8165	

Between both Groups A & B, the difference in mean Fibrotic villi, the mean Syncytial knots, the mean Vasculo syncytial membranes and mean Fibrinoid necrosis were observed to be statistically significant (p<0.0001).



**Fig. 3: Graphical representation of Histological parameters of both the groups**

From Fig. 3 it is observed that in hypertensive Group A, the mean Fibrotic villi of patients was 13.2000; the mean Syncytial knots of patients were 36.5600; the mean Vasculo syncytial membranes of patients were 5.0400 and the mean Fibrinoid necrosis was noted to be 10.2000.

In normotensive Group B, the occurrence of mean Fibrotic villi of patients was 5.0800; the mean Syncytial knots of patients were 20.6800; the mean Vasculo syncytial membranes of patients were 20.0800. the mean Fibrinoid necrosis of patients was noted as 5.0000.

Between both Groups A & B, the difference in mean Fibrotic villi, the mean Syncytial knots, the mean Vasculo syncytial membranes and mean Fibrinoid necrosis were statistically highly significant (p<0.0001).

**DISCUSSION:**

Many previously conducted researches have already shown important relation between maternal & fetal health with the structure of the placenta found in PIH mothers. In our study also we have found significant changes in placental morphology both macroscopic and microscopic; changes in fetoplacental weight ratio was also seen. Mardi K et al. [3] (2003) suggested a statistically significant raise in the occurrences of infarcts, intervillous deposits of fibrin, interstitial fibrosis and syncytial nodules in placenta with IUGR. Katzman PJ [4] (2002) observed that huge and dangerous number of fibrin deposits were more frequently seen in placenta with IUGR, than normal. Vander Veen F [5] (1983) suggested that a major factor in the cause of restricted fetal growth is amount of changes in the maternal spiral vessels, thus limiting the nutritional supply of the fetus. Egbor M [6] (2006) stated that the mean total volume of terminal & intermediate villi in placentas with IUGR was considerably lower than in control placentas. Biswas, Ghosh & Chhabra in their study (2008) [7] aimed to shed light on global morphological changes in the placenta that might contribute to increased incidences of idiopathic IUGR. According to Burton et al (2009) [8] a major reason for abnormal vascular remodelling & increased syncytial nodules might be formation of reactive oxygen compounds under high oxidative stress observed in hypertension in pregnancy. Similar results have been reported by Sankar, Bhanu & Kiran (2012) [9] in their study. Heazell & Martindale in their study (2009) [10] also quoted that abnormal placentas are related to still birth or IUGR. Burke & Globe in their research (2005) [11] proposed IUGR and placental infarction are strongly related to apoptosis. Rathod KB, Jaiswal KN (2007) [12] showed placental pathological changes in the form of increased infarcts, calcifications, and syncytial nodules. Wang ZJ, Yu YH (2002) [13] demonstrated interstitial fibrosis, fibrinoid necrosis, leukocyte villous infiltration, increased villous syncytial nodules and cytotrophoblast hyperplasia in the placenta of PIH complicated with IUGR showed pathological changes. Udainia A, Jain M [14] conducted a study on placental morphology in pregnancy-induced hypertension & its clinical relevance. The study was conducted in 75 gestational hypertensives and 25 normotensive pregnancies.

Fox (1975) [15] emphasized the importance of quantitative analysis of placental pathology, stating that the importance of lesions can only be identified if assessed by fetal growth and maturation. Bewley S et al. [16] performed a Doppler based study which showed in the second trimester there is increased resistance uteroplacental blood flow. Bhatia A et al. [17] conducted a comparative study to show similar placental and fetal outcomes in case of pregnancy induced high blood pressure.

Ghodke SP et al. [2] in the study titled Hypertension affecting morphometry of Placenta calculated the volume of placenta using Archimedes principle. Boyd PA, Scott A [18] gathered placentas from pregnancies with preeclampsia, essential hypertension, hypertension with preeclampsia, and normotensive pregnancies were examined by quantitative morphometry & findings were compared.

The objective of the current study was to highlight the pathological changes in placenta in pregnancy-induced hypertension (PIH) & correlation of the results with neonatal birth weight compared with normotensive mothers. A normal placenta weighs between 400 and 800 g. In the current study, placental weight varies between 306 and 604 g. The current study showed that there is a decrease in placental weight in hypertensive disease. For each placenta, the following measurements were recorded - weight, volume, central thickness, number of cotyledons, and fetal weight. In addition to the above measurements, histological differences between the two were also measured under the following parameters:

syncytial nodules, syncytial vascular membrane, fibrinoid necrosis, and fibrous villi.

The mean weight of placenta in the control group in this study was 485.85g compared to 509.5g observed by Majumdar S et al. (2005) [20]. In the present study, it was observed that the mean weight of placenta in hypertension was 414.44. According to the results of a research done by Chakravorty AP (1967) [23] mean placental weight was found to be 410 g for mild PIH and 350 g for severe PIH. The results are in accordance with Cambale T et al. (2016) [24] where the same findings also revealed that the mean weight of placenta in his PIH group (405.2 g) was lower than the control group (489.1g); also fetal birth weight also decreases with increasing PIH (control group was 2739.7g, whereas the PIH group was 2079.6g). In the present study, baby birth weight varied between 2250 and 3510 g in the control group, with a mean birth weight of 2950 g. On the other hand, in hypertensive pregnancies, it was 2688 g and fluctuated between 2090 and 3220 g. In the current study it was observed that newborn birth weight was less in case of hypertensive pregnancies and that this reduction was statistically significant.

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