HISTOMORPHOLOGICAL STUDY OF PLACENTA IN TOXAEMIA OF PREGNANCY
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ABSTRACT

BACKGROUND
Pregnancy-Induced Hypertension (PIH) also known as toxaemia of pregnancy is the most common complication during pregnancy. It is the leading cause of maternal and perinatal morbidity and mortality worldwide. The incidence is higher in developing countries with malnutrition, hypoproteinaemia and poor obstetric facilities.

The aim of the study was hence undertaken to analyse the effects of PIH on placenta as these changes serve as a guide to the duration and severity of disease.

MATERIALS AND METHODS
The study was conducted from September 2015 to August 2017 in Department of Pathology, MKCG Medical College, Berhampur (Odisha). The study was done in 104 placenta, 52 placentae from uncomplicated full-term deliveries formed the "control group" and 52 placentae from hypertensive pregnancies formed the "study group." All the placentae were studied morphologically and sections were taken from placenta and processed for histopathological examination.

RESULTS
The striking villous abnormalities studied were increased syncytial knots (82.7%), fibrinoid necrosis (67.3%), decreased villous vascularity (55.8%) and villous stromal fibrosis (59.6%). The gross abnormalities observed were decreased placental weight (94.2%), calcification (9.6%) and retroplacental haematoma (13.5%). Out of 52 normotensive placentae studied, the abnormalities observed were syncytial knots (15.4%), fibrinoid necrosis (3.8%), decreased villous vascularity (0%), villous stromal fibrosis (1.9%), decreased placental weight (11.5%), calcification (7.69%) and retroplacental haematoma (0%).

CONCLUSION
The gross abnormalities and villous lesions in PIH (p <0.001) were statistically significant.

KEYWORDS
Pregnancy-Induced Hypertension, Villous Abnormalities.


BACKGROUND
Placenta is a unique and wonderful organ said to be the most accurate record of infant's prenatal experience.¹ A foetus, a placenta and a mother form a composite system of dynamic equilibrium.² The placenta acts as a "diary of intrauterine life", which has potency of focussing the development process going during the pregnancy.³ Placenta was often regarded as the waste product of the birth process, but obstetricians consider it to be the most important foetal organ. Being an organ of vital importance for continuation of pregnancy and foetal nutrition, it has evoked great interest among the obstetricians as well as pathologists to understand the unique biological status of this complex organ.⁴ Any pathology of placenta has direct hazardous impact over foetal outcome. Still, it continues to be an underutilised and improperly handled surgical material.⁵

Toxaemia of pregnancy that includes pre-eclampsia and eclampsia is a pregnancy-specific multisystemic disorder that manifest after 20 weeks of gestation⁶ and is characterised by hypertension, proteinuria, oedema and platelet aggregation.

Hypertensive disorders complicate about 3-10% of all pregnancies. PE and related conditions are among the leading causes of maternal mortality worldwide.⁷ A study of the Indian population found the prevalence of hypertensive disorders of pregnancy to be 7.8% with pre-eclampsia accounting for 5.4%.⁸ Similarly, the incidence of complications such as eclampsia is about 50 per 10,000 per year in low-economic countries, which is 10 times that in the developed world.⁹ Pregnancy complicated by hypertension is commonly associated with placental insufficiency.¹⁰ It has been recorded that maternal uteroplacental blood flow is decreased in pre-eclampsia because there is maternal vasospasm.¹¹ So, the present study was undertaken to compare the morphological and histological changes in the placenta of normal and hypertensive mothers.
MATERIALS AND METHODS
The present study was conducted in the Department of Pathology, M.K.C.G. Medical College, Berhampur, during the period from September 2015 to August 2017. The study was done in 104 placentae, 52 placentae from uncomplicated full-term deliveries formed the "control group" and 52 placentae from hypertensive pregnancies formed the "study group."

Inclusion Criteria
Pregnant women of more than 20 weeks gestation having BP >140/90 mm of Hg and above with or without oedema and/or proteinuria were included.

Exclusion Criteria
Cases like gestational diabetes mellitus, lupus antiphospholipid syndrome, chronic hypertension and maternal thrombophilias were excluded from the study. Also, women having hypertension before pregnancy were excluded.

Method of Sample Collection
- Placentae with umbilical cord and attached membranes were collected soon after delivery. The umbilical cord was cut at a distance of 5 centimetres from the site of attachment. Placentae were drained completely of blood, washed with water and weighed before formalin fixation on a weighing machine (g). The mode of insertion of the cord were ascertained. Then, the placenta was examined for gross abnormalities. The gross abnormalities were quantified using semiquantitative method as "absent" (no visible lesion), "+" (locally distributed lesions) and "++" (extensive lesion). All placentae were cut along maximum visible lesion), all placenta were cut along maximum diameter in two equal halves and then were further cut in small pieces. Two sections each from central and peripheral areas were taken. Additional sections were taken from grossly abnormal lesions. Sections were stained with the Haematoxylin and Eosin (H and E) stain. One hundred villi were counted from each of the four sections obtained and histological changes expressed as percentage. In addition, depending on the need, special stains like Periodic Acid-Schiff (PAS) and Masson’s trichrome stains were used to highlight the membrane abnormalities and elastic tissue.

Statistical Analysis
- The incidence of various gross and histological features was compared with that of normal pregnancies by using the unpaired-type Student’s t-test. A ‘p-value’ of less than 0.05 was considered to be statistically significant.

RESULTS
The present study included 104 placentae, 52 placentae each of control and study group. Gross morphological features and microscopic features in the present study among both groups are compared in Table 1 and Table 2, respectively.

The majority of cases of toxaemia of pregnancy in the present study were found in younger age groups (80.8%).

Both primigravida and multigravida were considered in the study and maximum cases (65.4%) was observed in the primigravida.

The present study reveals significant low foetal weight in the hypertensive group (<2.5 kg weight in 63.5%) than the control group and weight of placenta shows significantly lower values in the study group than in the control normotensive group.

The average weight of placenta in hypertensive pregnancies was less than 500 g, the least weight recorded being 180 g, whereas average placental weight of normal pregnancies was more than 500 g and the heaviest being 540 g.

<table>
<thead>
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<th>Features</th>
<th>Study Group</th>
<th>Control Group</th>
<th>P value</th>
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<tbody>
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<td>Frequency</td>
<td>Percentage</td>
<td>Frequency</td>
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<tr>
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<td>0</td>
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<tr>
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<td>8</td>
<td>15.4</td>
<td>0</td>
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<tr>
<td>Calciﬁcation</td>
<td>5</td>
<td>9.6</td>
<td>4</td>
</tr>
<tr>
<td>Retroplicental haematoma</td>
<td>7</td>
<td>13.5</td>
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Table 1. Gross Morphology of Placenta

Majority of the placenta were round in both groups. The commonest mode of insertion of the umbilical cord into the placenta was central (65.4%), followed by eccentric (28.8%) and marginal insertion (5.8%) being the least.

Placental infarct appears ﬁrm and dark red in colour. As it turns older, it becomes hard white mass with granular appearance. In the present study, the incidence of significant placental infarction (>5% surface area involved) was 15.4% in the study group. Calciﬁcation was seen in 9.6% cases of study group as compared to 7.69% cases of control group. Retroplicental haematoma was noted in 13.5% of cases in study group and none of the control showed this.

<table>
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<th>Features</th>
<th>Study Group</th>
<th>Control Group</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
<td>Frequency</td>
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<tr>
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<td>34.6</td>
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</tr>
<tr>
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<td>55.8</td>
<td>0</td>
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<tr>
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<td>5</td>
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Villous abnormalities seen histopathologically among both groups are compared in Table 2. Marked villous hypovascularity (55.8%) and hypervascularity (9.6%) was seen respectively in hypertensive cases.

Increased syncytial knot formation seen as focal aggregates of syncytial nuclei forming a multinucleated protrusion from the villous surface was seen in 82.7% of placentae of toxemia of pregnancy and 15.4% of control group. Highly significant association was observed between the two groups.

In this study, there was a higher percentage of stromal fibrosis (59.6%) in toxaeamic cases and in 1.9% of control group and the two group showed highly significant association (p=0.000). Fibrinoid necrosis of terminal villi is a highly characteristic lesion. Fibrinoid necrosis was seen in HE stained sections as small nodules of homogenous eosinophilic material within the villi. In the present study, fibrinoid necrosis was observed in 67.3% of toxaeamic cases and 3.8% of normotensive control group respectively and was statistically significant (p=0.000). Vasculosyncytial membrane was seen in the villous as attenuated areas of syncytiotrophoblast, which overlaid and appeared to fuse with the wall of the adjacent dilated foetal capillary. Vasculosyncytial membrane in less than 5% of villi was considered significant and was observed in 17 cases (32.7%), while none of the control showed significant vasculosyncytial membrane deficiency (p < 0.000).

**DISCUSSION**

The placenta is a highly specialised organ of pregnancy described as an effective index for maternal and foetal status in pregnancy. Placenta brings the mother and foetus, the two important ends of reproduction in contact with each other. Therefore, the placenta, which is usually considered as records of infant’s prenatal experience provides crucial information about the deleterious effects of pregnancy-induced hypertension on foetal outcome.

The majority of cases of toxaeama of pregnancy in the present study were found in younger age groups (80.8%) and in primigravida (65.4%). This has also been observed by Kher et al (1981) and Dutta et al (1989).12,13 According to Page (1972), primigravida have unyielding abdominal wall leading to higher intra-abdominal pressure that could lead to decreased uterine blood flow by external compression of myometrium as the uteroplacental blood flow in part is controlled by tone of myometrium through, which all blood vessels must pass to reach the intervillous space.

The present study reveals significant low foetal weight in the hypertensive group (<2.5 kg weight in 63.5%) than the control group and weight of placenta shows significantly lower values in the study group than in the control normotensive group. These findings corroborate with the studies of other workers like Damania (1989), Fox (1994) and Kalousek (1994). According to Younoszai and Haworth (1969), placental weight and size were directly proportional to the birth weight of the babies. Rath in 1994 stated that in hypertension arrangement of intracotyledonous vasculature is altered resulting in low birth weight of babies. Similar findings were present in study done by other workers like Ahmed M et al (2013), Sreechithra Kartha et al (2014) and Kumari V et al (2016).14,15,16

The average placental weight is less in study group, (<500 g in 94.2%), the least weight recorded being 180 g. Similar results were reported by Udaina et al, Bazaz et al and Mujumdar etal17,18,19 who found reduced placental weight in pre-eclamptic cases as compared to normotensive controls. Comparative studies were also done by Narasimha A.20 Cibils (83) reported that placenta from PIH cases were smaller than normal indicating an underlying pathological process interfering with the normal growth of placenta. The main reason for reduced placental weight in pre-eclampsia could be uteroplacental insufficiency. It has been recorded that maternal vasoospasm in pre-eclampsia leads to decreased maternal uteroplacental blood flow. Hypoxia and reduction in blood flow could be responsible for morphological alterations of placenta in pre-eclampsia. Decreased placental weight in pregnancy-induced hypertension was also found by other author like P. Anjankar et al (2014), L. Singhal et al (2015) and Avinash Chandra et al (2016).21,22,23

In the present study, among hypertensive group, three placentae showed marginal insertion of cord, whereas central and eccentric insertion of cord seen in 34 and 15 number of cases, respectively. The placentae of the normotensive group mostly showed central insertion (80.8%) of umbilical cord. There is significant association between insertion of umbilical cord and hypertension in pregnancy (p=0.05) in this study. Siva Sree Ranga et al (2017) observed five placenta with abnormal insertion of cord. Among them, four placentae showed marginal insertion and one showed velamentous insertion, while the placentae of normotensive group showed central insertion of umbilical cord. There was insignificant association between insertion of umbilical cord and hypertension in pregnancy in their study.24

Placental infarction represents an area of ischaemic villous necrosis secondary to local obstruction of the maternal uteroplacental circulation. Minor degree of infarction is seen in about 25% of placentae from uncomplicated pregnancies and can therefore be regarded

<table>
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<th>Syncytial knots</th>
<th>Normal</th>
<th>9</th>
<th>17.3</th>
<th>44</th>
<th>84.6</th>
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<tbody>
<tr>
<td>Increased</td>
<td>43</td>
<td></td>
<td>82.7</td>
<td>8</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>31</td>
<td></td>
<td>59.6</td>
<td>1</td>
<td>1.9</td>
<td></td>
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<tr>
<td>Fibrinoid necrosis</td>
<td>Present</td>
<td>35</td>
<td>67.3</td>
<td>2</td>
<td>3.8</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>17</td>
<td>32.7</td>
<td>50</td>
<td>96.2</td>
<td></td>
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<tr>
<td>Vasculosyncytial membrane</td>
<td>Normal</td>
<td>35</td>
<td>67.3</td>
<td>52</td>
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<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Decreased</td>
<td>17</td>
<td>32.7</td>
<td>0</td>
<td>0</td>
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</table>

**Table 2. Microscopic Features of Placenta**
as an inconsequential phenomenon. Infarction involving more than 5% of placental parenchyma is clinically significant. In the present study, there is significant infarction in 15.4% of hypertensive cases and is absent in normotensive group and the observed difference between the two groups is statistically highly significant (p=0.003). Siva Shree Ranga et al (2017) observed infarction in 36.7% of hypertensive cases and 6.7% of non-hypertensive cases.24

The incidence of calcification in the present study is 9.6% in hypertensive group and 7.69% in normotensive group, respectively, and the observed difference between the two group is statistically significant (p <0.05). Placental calcification was seen more commonly in placentae from hypertensive patients. Calcification is regarded as evidence of placental ageing or maturation. A correlation between placental calcification and primigravidity has been noted by Pasricha Navbir (2012).25

Retrolaplacental haematoma is significant if more than a third of the placental parenchyma is separated by the haematoma from the maternal utero-placental vessels. Navbir P noted retrolaplacental haematoma in 30.52% of pregnancy-induced hypertension cases.26 In the present study, we observed 13.5% retrolaplacental haematoma in hypertensive cases, while no retrolaplacental haematoma in control group. Some worker stated retrolaplacental haematoma as a complication of hypertensive disorder of pregnancy like Bartholomew and Kracke, while others stated retrolaplacental haematoma as a cause of hypertensive disorder of pregnancy like Hibbard and Jeffcoat.

Wide spectrums of villous lesions were observed in toxemic cases in the present study. The terminal villi of the mature placenta usually contain 2-6 capillaries. The possible deviations from this vasculature are encountered, namely villous avascularity, hypovascularity and hypervascularity. In the present study, the incidence of villous hypovascularity (55.8%) was increased in the hypertensive group in comparison to the control group where the incidence of normal villous vascularity was more. Krielessi et al observed reduced number of vessels in pre-eclamptic placentae. Reduced number of vessels maybe due to their reduced formation or secondary to stromal fibrosis.20 Coelho et al observed poor microvessel density in hypertensive pregnancies, which worsened with increasing levels of hypertension and proteinuria. This shows decreased vascularisation with increased severity of disease, which consequently leads to lower nutritional status of foetus.

Other villous lesion like increased syncytiotrophoblast formation seen as focal aggregates of syncytiotrophoblast nuclei forming a multinucleated protrusion from the villous surface was seen in 82.7% of placentaes of toxemia of pregnancy and 15.4% of control group. Highly significant association was observed between the two groups. Syncytiotrophoblast plays a major role throughout pregnancy and is a major site for nutrient exchange. Aggregates of syncytiotrophoblast nuclei on the surface of terminal villi are called as syncytiotrophoblast. The cells of both cytotrophoblast and syncytiotrophoblast divide ultimately and undergo apoptosis. The number of syncytiotrophoblast nuclei increases 9 fold from 13 weeks of gestation to term and can be used to evaluate villous maturity. Most of these nuclei are dispersed within the syncytiotrophoblast, while old apoptotic nuclei aggregate and form membrane sealed bodies that are referred as syncytiotrophoblast nuclei. Pathogenic mechanism proposed behind pre-eclampsia is placental ischaemia and hypoxia, which may affect the trophoblast functioning and lead to stimulation of apoptotic pathway thus increasing rate of trophoblast turnover. Tenny and Parker (1940) quoted that the influence of maternal factors is best shown in pre-eclampsia where the decreased intervillous blood flow finally leads to increased syncytiotrophoblast knots, so called the Tenny-Parker changes. He emphasised that the increased bridging of placental syncytiotrophoblast nuclei is very much characteristic of pre-eclampsia. Syncytial knots are also seen normally in term and preterm placentae, but their number is much increased in toxemia.

In the present study, there was a higher percentage of stromal fibrosis (59.6%) in toxemic cases and in 1.9% of control group and the two groups showed highly significant association.

The two factor responsible for the formation of stromal fibrosis are a normal ageing process and a reduced utero-placental blood flow.20 However, insignificant association between stromal fibrosis and foetal outcome was reported by Kher et al25 and Navbir et al25 found stromal fibrosis commonly in control placentae as well as pre-eclamptic placentae. According to them, stromal fibrosis is also commonly found in control placentae and does not seem to affect the foetal outcome.

In the present study, fibrinoid necrosis was observed in 67.3% of toxemic cases and 3.8% of normotensive control group respectively and was statistically significant. Fibrinoid necrosis of terminal villi is a highly characteristic lesion. Fibrinoid necrosis was seen in HE stained sections as small nodules of homogenous eosinophilic material within the villi. At places, the fibrinoid had enlarged pushing the basement membrane and compressing the entire villous stroma. Majumdar et al25 also observed a significant increase in number of terminal villi with fibrinoid necrosis with mean diastolic blood pressure 110 mm of Hg. Kurdukar et al mentioned same finding in severe pre-eclampsia.8 Bukhari et al observed increased number of terminal villi manifesting fibrinoid necrosis with an increase in diastolic pressure. Narasimha and Vasudeva also noted significant villous fibrinoid necrosis in 97.82% cases of the pre-eclampsia.10 This increased deposition of fibrinoid was initially thought due to elevated blood pressure, but it is now believed that it is due to inappropriate immune response. Burstein et al have found that fibrinoid necrosis is a form of senile amyloid due to immune attachment on trophoblastic cells, which because of ageing process contains misspecified proteins.

Paucity of the vasculosyncytiotrophoblast membrane is an index of foetal hypoxia. Vasculosyncytiotrophoblast membrane was seen in the villous as attenuated areas of syncytiotrophoblast, which overlaid and appeared to fuse with the wall. It is formed when syncytiotrophoblast surrounds the terminal villi and
makes close contact with the foetal capillaries. Sinusoidal dilatation of terminal villous capillaries bulging against the trophoblastic surfaces and their alteration to a thin lamella results in vasculosyncytial membrane. It is closely related to foetal villous vascularisation. Immature placenta show reduced villous carilisation and paucity of vasculosyncytial membrane.

Vasculosyncytial membrane in less than 5% of villi was considered significant and was observed in 17 cases (32.7%), while none of the control showed significant vasculosyncytial membrane deficiency (p <0.000). Vasculosyncytial membrane deficiency was significant in the study group comparable to finding by Fox (1967) who reported a low vasculosynitial membrane count (4.5-5.3%) in hypertensive placentae and considered it to be a manifestation of villous regression. Fox (1968) considered that the reason for higher percentage of villi with thickened basement membrane is due to the proliferation of cytotrophoblastic cells, which secrete the basement membrane as a response to placent ischaemia. The present study well correlated with the observation of Kher et al (1981), Mohan et al (1990) and V. Porwal et al (2017).12,26,27

CONCLUSION
Placenta serves as a very valuable diagnostic tool to understand and explain the pathophysiology of various conditions affecting the mother, child or itself.

Standardisation of the method of placental examination is essential to achieve optimal benefits from the diagnostic reports. The placental changes are essential to correlate the foetal outcome as it provides the information for the cause of death. Hence, it has an effective role in planning prenatal monitoring of a future pregnancy. A thorough and proper study of placenta coupled with complete clinical details will certainly enrich our knowledge to ensure better maternal and neonatal care.

REFERENCES


