Original Article

Statistical Analysis Of Human Placental Histological Changes In Mild, Severe Prgegnancy Induced Hypertensive Conditions And Normotensive Pregnancy Using Chi-Squared Test

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Abstract

Background: Pregnancy induced hypertension (PIH) is one of the most common complication during pregnancy. PIH is strongly associated with both maternal and foetal complications. Current study was undertaken to analyze placental changes in the mild and severe PIH in order to understand the importance of placental changes in understanding the severity of disease and complications. After studying all the placental findings, it was evident that the calcifications, retroplacental hematomas, villous hyper maturity and fibrin deposition were more prominently related to severe hypertensive cases compared to mild cases of PIH. Moreover, it could complicate pregnancy adversely by causing histopathological changes in the placenta of severe PIH cases than in mild PIH cases along with poor foetal outcome.

Objectives: Comparative evaluation of histopathological changes of placenta of women with mild and severe PIH with that of normotensive pregnancies.

Materials and Methods: A total of 150 pregnant women (50- normotensive,50-mild and 50-severe PIH) between the age group of 18-35 years, gestational age between 26-42weeks, attending antenatal clinic of the hospital and delivered by either vaginal or caesarean section were included in the study after taking the written informed consent from the participants. Standard techniques were used for histological preparation and staining as specified in the literature.

Results:Pregnancy-induced hypertension(PIH) adversely affects the health of the foetus through its harmful effects on the placentas. The observations from the present study found that there was a significant difference between the calcifications, RPH, FD, TPC, AV, VHM of mild and severe PIH cases with that of normotensive pregnancy cases.

KEYWORDS: Comparative Study, Placental histological Changes, Mild and severe Pregnancy induced Hypertension.

INTRODUCTION

The placenta is a fetomaternal organ.It is a connection of the foetus with the uterine wall of mother. The placenta is essential for maintenance of pregnancy and for promoting normal growth and development foetus. It is the most accurate record of foetus. Pregnancy induced hypertension (PIH) is common and form a deadly triad beside

hemorrhage and infection that contributes to maternal mortality and morbidity. Their incidence in pregnancy is 5% - 10% [1] Women in developing countries are seven times more likely to develop preeclampsia than women in developed countries[2]. Maternal mortality rate (MMR) in India is 179 per one lakh live births[3]. PIH are strongly associated with both maternal and foetal complications. Placenta which is an essential organ maintaining pregnancy and promoting foetal development, which functions as to help developing foetus to derive its nutritional substances and obtain its metabolic and immunological requirements[4].

The etiology and pathological mechanism in the development of pregnancy hypertensive disorders is still controversial. However, placenta is the key organ in pathogenesis of the disease and important mechanism involved in defective remodeling of spiral arteries that could contribute to the hypoxic environment and thereby placental insufficiency which can lead to the maternal and foetal complications[5,6].The maternal and foetal complications in hypertensive disorders complicating pregnancy are associated with significant pathological variations in placenta depending on severity of hypertension (HTN), whether it is mild or severe PIH[7]. Placental examination will help in understanding the specific etiologies of adverse outcome, which will lead to specific preventive and treatment measures for those with the risk for recurrence in further pregnancies along with long-term sequelae in pregnancy hypertensive disorders. The present study is undertaken to analyze Variation of placenta in the mild and severe PIH which helps to analyze importance of placental changes and helps to understand the severity of disease and complications.

MATERIALS AND METHODS

STUDY DESIGN:The present study was a Comparative descriptive study with analytical components. The study was conducted at Government Medial college and Hospital, Srikakulam, Andhra Pradesh, during period from june 2019 to December 2020.

Sample: A total of 150 pregnant women (50-normotensive,50-mild PIH and 50-severe PIH) between the age group of 18-35 years, gestational age between 26-42weeks, attending antenatal clinic of the hospital and delivered by either vaginal or caesarean section were included in the study after taking the written informed consent from the participant.The study protocol was approved by the institutional Human Ethical Committee (IHEC) of Government medical college and Government General hospital,Srikakulam, Andhra-Pradesh, India, conducted on 25th June 2019: approval number ECR/492/Inst/AP/2013/RR.

SELECTION CRITERIA:

Inclusion criteria:

Group1: Healthy controls were the individuals with no history of high blood pressure (hypertension) and with no evidence proteinuria or any other complications prior to the pregnancy. Normal blood pressure for most adult is defined as a systolic pressure of 120 and a diastolic pressure of 80.

Group2: Mild Hypertensive women defined and taken as the BP elevation of ≥140/90 mm of Hg by sphygmomanometer.

Group 3: Severe hypertension defined and taken as BP elevation greater than 160/110 mmHg.

Exclusion Criteria: Antenatal women with partial molar pregnancy, antenatal women with multiple pregnancy, Pregnant women with any other complications such as diabetes mellitus, hypothyroidism, anaemia, abruption placentae, multiple pregnancies and jaundice during pregnancy.

PARAMETERS INVESTIGATED:

Blood Pressure (BP) measurements were obtained by a mercury sphygmomanometer in all subjects, three consecutive times with a gap of five minutes. Korotkoff phase five was used to designate diastolic blood pressure. In cases where BP was consistently higher in one arm, the arm with the higher values was taken for all BP measurements. The mean of three systolic values and the mean of three diastolic values of the same patient were taken as mean of systolic blood pressure (SBP) and the mean of diastolic blood pressure (DBP) respectively for

an individual. Pregnant women grouped under Mild hypertension (HTN) when their blood pressure level was 140-159 mmHg systolic and 90-99 mmHg diastolic [8]. Similarly, pregnant women were grouped under severe hypertension (HTN) when their pressure was elevated >160/110 mmHg.

Histo-pathological findings of placenta: Calcifications, retro-placental hematoma (RPC), infarctions, tenny-parker changes(TPC), avascular villi(AV), villous hyper maturity(VHM), fibrin deposition(FD) were evaluated. Standard techniques were used for Histological preparation and staining as specified in the literature.

Collection of placenta sample and Tissue preparation for routine H&E staining:

Placenta along with the umbilical cord was obtained immediately after the delivery from 50 normotensive, 50 mild and 50 severe PIH women, whose deliveries were normal or through caesarian sections.Collecting the placental sample 3 cm away from the central placental disc. Only one cotyledon was selected and fragmented to smaller parts for the proper fixation of the sample which is about 2-3mm thickness. Sample fixation was done by using 10 % neutral- buffered formalin solution. Within 24 hours after sample fixation further processing of the sample was done for staining procedures. These samples are processed.

RESULTS

The frequency distribution of calcifications, RPH, FD are shown in (Table 1.1).The normal category showed 10% calcifications, whereas the mild category showed 28% calcifications, severe showed 68% calcifications. This is statistically significant (P<0.001). The normal category does not show RPH. whereas mild category showed 24%, severe category showed 44% RPH. This is statistically significant (P<0.001) The normal category does not showed FD, whereas mild category showed 34%,severe category showed 64% FD. This is statistically significant (P<0.001).

The frequency distribution of Infarctions, TPC, AV, VHM, are shown in (Table 1.2) The normal category showed 1% infarctions, whereas mild category showed 18%, severe category showed 30% infarctions. This is statistically significant (P<0.001) The normal category does not show TPC, whereas mild category showed 50%, severe category showed 68% TPC. This is statistically significant (P<0.001) The normal category does not show AV, whereas mild category showed 30%, severe category showed 48% AV. This is statistically significant (P<0.001) The normal category does not show VHM, whereas mild category showed 36%, severe category showed 56%VHM.This is statistically significant (P<0.001).

Table 1.1 : Comparison of Histo-pathological findings in normal, mild and severe PIH pregnancies

S. No.	Variable	Groups	Category		Statistical analysis
			Absent	Present	
1	Calcification	Control	45	5	$\chi 2 = 38.572$
		PIH-mild	36	14	$P < 0.001$
		PIH-severe	16	34	
2	Retro Placenta Haematoma	Control	50	0	$\chi 2 = 27.688$
		PIH-mild	38	12	$P < 0.001$
		PE-severe	28	22	
3	Fibrin deposition	Control	50	0	

		PIH-mild	33	17	$\chi 2 = 46.615$
		PIH-severe	18	32	$P < 0.001$

Table 1.2 : Comparison of Histo-pathological findings in normal, mild and severe PIH pregnancies

S. No.	Variable	Groups	Category		Statistical analysis
			Absent	Present	
1	Infarctions	Control	49	1	$\chi 2 = 14.208$ $P < 0.001$
		PIH-mild	41	9	
		PIH-severe	35	15	
2	Tenny Parker changes	Control	50	0	$\chi 2 = 73.891$ $P < 0.001$
		PIH-mild	25	25	
		PIH-severe	16	34	
3	A vascular villi	Control	50	0	$\chi 2 = 30.561$ $P < 0.001$
		PIH-mild	35	15	
		PIH-severe	26	24	
4	Villus Hyper maturity	Control	50	0	$\chi 2 = 37.876$ $P < 0.001$
		PIH-mild	32	18	
		PIH-severe	22	28	

DISCUSSION

Placenta is a specialized structure that supplies the nutrients and other essential substances from mother to the foetus. Placental hemodynamics is affected in the pregnant women with PIH. Along with the hemodynamic changes there may be structural changes in the placenta. These effects the other systems of the body and eventually effects the homeostasis of both mother and foetus. Digital images of the tissues were captured using low power and high power fields of Olympus DP-20 color video camera interfaced with an Olympus CX 2LI microscope. For microscopic examination sections were stained with hematoxylin and eosin stain, for study of calcifications, retro placental hematoma (RPH) infarctions, tenny-parker changes (TPC) avascular villi (AV), villous hyper maturity (VHM), fibrin deposition (FD) among mild and severe PIH and normotensive placenta. The observations correlate well with the previous studies.

Calcifications would be visible on gross examination. Fibrin or fibroid material containing placentas often shows calcifications. They produce a multifocal blue colour with hematoxylin and eosin staining. Low utero-placental blood flow may be the cause for these changes. Severe and diffuse calcification often associated with the hypoxic new-born. The calcifications in the placenta were associated with the severity of the hypertension. In one study, placentas of mild hypertension, and severe hypertension were compared[9]. They observed that calcifications were more in the severe hypertension group than the mild group, but that was not statistically significant in their study. In another study[10]studied placenta findings were correlated with the severity of the hypertension. They found that calcifications were more in the severe group than the mild hypertensive group. These results were similar to the present study. From the observations made in the above studies, it was found that calcifications were more common in placentas of women with severe hypertension.

Retro placental hematoma is a clot located between the basal plate of the placenta and the uterine wall. These are less common, occurring in about 12-15 % all PIH cases and 4.5 % of all placentas. Microscopically this change is characterized by red cells and fibrin. As the lesion age increases proportion of fibrin increases and red cells degenerate. They are associated with high incidence of foetal hypoxia and death. In the present study, incidence of RPH was observed in mild hypertensive group where as in severe group, but there was an increased incidence seen in severe hypertensive group and was statistically significant. This shows that RPH, as one of the gross examination finding in the placenta, associated with the severe hypertensive disorders of pregnancy. In another study placenta were divided into groups, depending on severity of the hypertension, observed that RPH found in severe group than mild hypertensive group [11]. It can be concluded that RPH, related to the severity of the hypertension.

Infarcts are numerous, larger (>1 cm3) and centrally located. A fresh placental infarct is well demarcated, dark red (< 2-3 days). As the infarct ages, it becomes progressively hard and the colour changes successively to brown yellow and white (after 1 week). An old infarct thus appears as a hard white plaque, with a smooth or slightly granular, amorphous cut surface. Histologically, the early infarct is characterized by aggregation of villi in the affected area, with marked narrowing and often obliteration of the inter-villous space. Infarction is associated with significant perinatal morbidity and mortality, including Intra uterine foetal death, foetal hypoxia, and neonatal morbidity and mortality. In the present study, the incidence of placental infarction was (30%) in severe hypertensive group as compared to only (18%) in the mild hypertensive group. In the present study it was observed that out of the total cases of placental infarction there was a relatively higher preponderance of infarction in cases of severe hypertension when compared to cases of mild hypertension complicating pregnancy and was statistically significant between these groups ($p<0.001$). In another study,55 placentas of subjects with mild hypertension and 55 placentas of subjects with severe hypertension reported that, women with severe hypertension had significantly higher (72.72%) incidence of placental infarction when compared to only (32.72%) in the mild hypertensive group [12]. In this study this variable was found to be statistically significant ($p <0.001$) From this study it was observed that infarction was related to the severity of the hypertension. In another study, it was observed that Infarction was seen in more number of cases of severe PIH group than the mild hypertensive group [13].

Syncytial knots can be seen in normal placenta but more than 30 % are indicative of per-fusional compromise. They are more significant when they are present in the central portion of the placenta, because peripheral portion has already low placental blood supply. When the villi are often small with excessive syncytial knots, it is known as tenny - parker changesIn the present study, it was observed that there is an increased incidence of TPC in placenta in the severe form of hypertension complicating pregnancy (68%) when compared to mild hypertension complicating pregnancy (50%) and the association was statistically significant (P value <0.001). A study with 200 case of 100 mild and 100 severe preeclampsia cases, observed that significantly increased number of TPC (syncytial knots) in the severe cases (46.7±11.81) than the mild cases (31.90±11.0),p value= 0.001 which was significant [14].

Avascular villi were diagnosed when a group of at least 5 fibrotic avascular villi without inflammation and without mineralization were seen. AV noted in placenta from foetus of low birth weight. In the present study, the incidence of AV was more in severe hypertensive group than the mild hypertensive group and was statistically significant. In a study the placentas of mild and severe hypertension was compared and noted the presence of avascular villi more in the severe group compared to the mild group, however, it was not statistically significant [15].

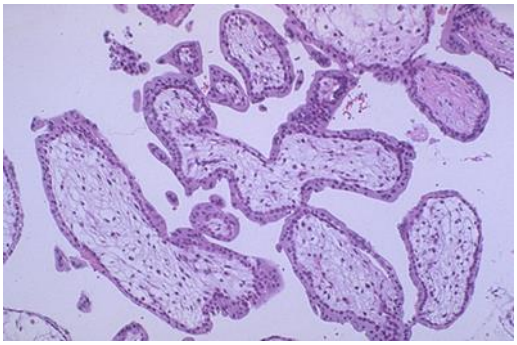
In the present study it was observed that there was a higher incidence of villous hyper maturity of the placenta in cases of severe PIH (56%) as compared to the mild hypertensive group (36%).There was statistically significant association of this variable with severity of the hypertension when compared between these groups. In another study conducted in two groups of mild,severe cases of hypertensive disorders of pregnancy observed that VHM was significantly higher in severe hypertensive group (40%) when compared to the mild group and this association was statistically significant. From this study it was clear that VHM is correlated with the severity of the hypertension.

fibrin and thrombotic material under the foetal surface. The eventual outcome of the foetal activity is associated with the sub-chorionic deposition of fibrin. When this deposition of fibrin surrounds more than half of the villous tissue it is considered to be massive inter villous hyper-maturity. In maternal floor infarction, there is excessive fibrin deposition at the maternal floor around the basal villi. This condition is most often associated with still birth, growth retardation and preterm delivery with possibility of recurrence in subsequent deliveries. In the present study, the incidence of FD was 64% in the severe group where as 34% in the mild hypertensive group. When compared the association between the two groups, it was found to be statistically significant. In another study the fibrin deposition was associated with severe hypertensive group than the mild hypertensive group [16]. Another study also has reported an increased incidence of fibrin deposition in the hypertensive group when compared to the normal group but no differentiation was made between the mild and severe groups [17].

Hypertension complicating pregnancy is commonly associated with low birth weight. The placental changes associated with hypertension complicating pregnancy were tabulated and compared individually with low birth weight and normal weight babies. From these observations it was found that calcifications and avascular villi of the placenta were significantly associated with low birth weight foetus. Despite the understand and benefits of examination of placenta, great resistance still exists in performing placental examination routinely therefore the placenta has remained neglected and mysterious organ. The placental examination will help in understanding of the specific aetiologies of adverse outcome which will lead to specific treatment and preventive measures for those with risk for recurrence in subsequent pregnancies and long term sequelae in hypertensive disorders of pregnancy.

The present study was undertaken to analyse placental changes in the mild and severe hypertensive disorders in pregnancy with a view to assess the significance of placental changes by histopathological methods because these changes may serve as a guide to the severity of disease .As placenta is the major nutrient source of the foetus, any damage to the structure of the placenta affects the nutrient supply of the foetus hence, it is the need of time to understand the importance of the histopathology of damage of placenta and its effects on the foetus [18].It was reported that due hypoxia which results from preeclampsia causes decrease in the vascularity of the placenta[19].The placental vascularity also influenced by the hormonal factors such as placental growth factors, insulin like growth factors[20]

The present study evaluated the pathological changes [Figure1.1,1.2,1.3]. The study results are in accordance with another study where the placenta of the mothers suffering with pre-eclampsia showed similar morphological changes as observed in the present study [21]. The perinatal morbidity associated with this condition is probably related more to some alterations in utero-placental, and possibly, umbilical blood flows than to significant changes in placental structure and function. In accordance with this study it can be concluded that hypertensive disorders complicating pregnancy adversely influence the pathological changes like incidence of calcification, RPH, VHM, FD were significantly higher in severe compared to mild PIH. The other histopathological changes like infarction, TPC, AV were increased in severe hypertensive disorders compared to mild hypertensive disorders and foetal outcome was poor as the severity of the disease increased. The present study was undertaken to analyse placental changes in the hypertensive disorders in pregnancy with a view to assess the significance of placental changes by histopathological methods because these changes may serve as a guide to the severity of disease. The placental examination will help in understanding of the specific aetiologies of adverse outcome which will lead to specific treatment and preventive measures for those with risk for recurrence in subsequent pregnancies and long-term sequelae in hypertensive disorders of pregnancy.



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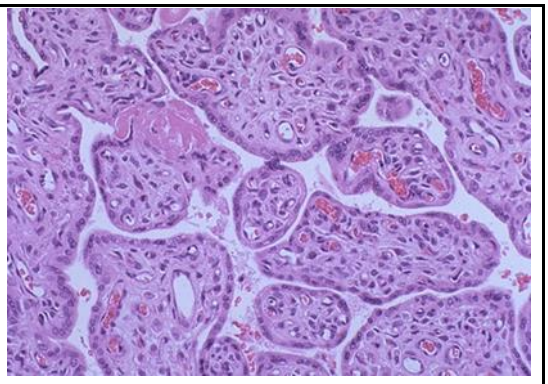

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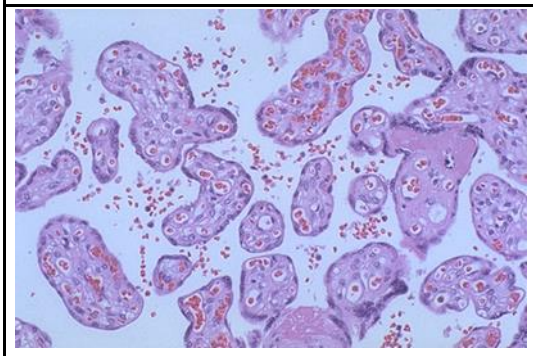

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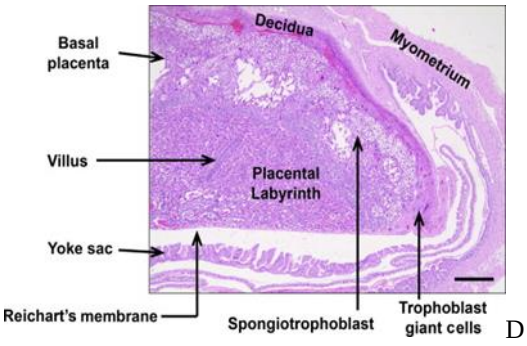
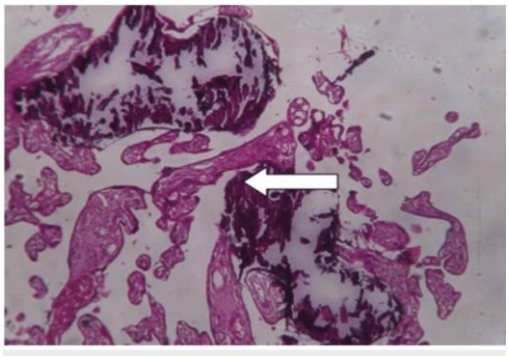


D

Figure 1.1 Representation of microscopic images of normal placenta.
a) In the first trimester placenta covered by two layers of cells, cytotrophoblast and syncytial trophoblast. The blood vessels in the villi are not prominent.
B) As the placenta matures and increases in size in the second trimester, the villi become smaller and more vascular.
c)A mature placenta in the third trimester has small and highly vascularized chorionic villi.
d)matured placenta.



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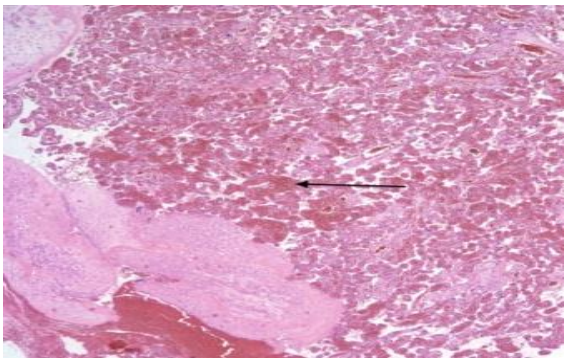

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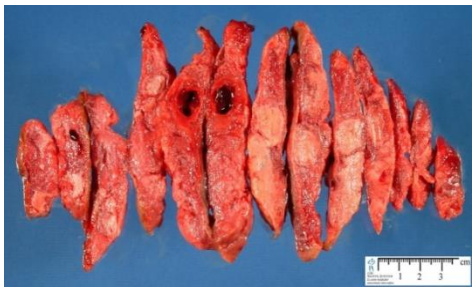

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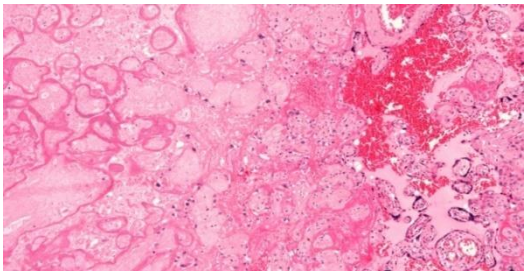
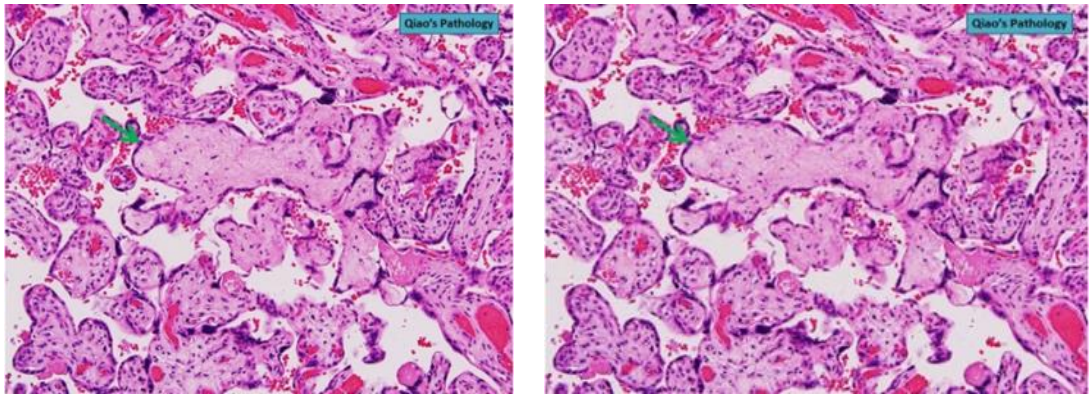


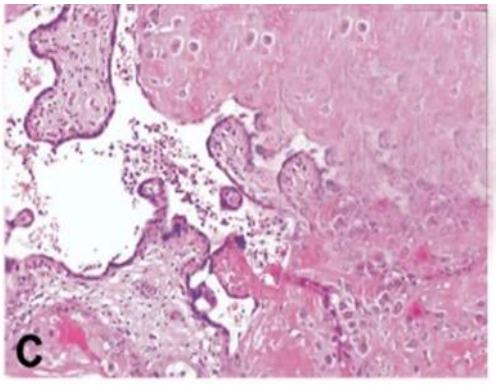
D

Figure 1.2: Representation of microscopic images of mild, severe PIH placenta.

a) Calcification b) Retroplacental heamotomac) Infarctiond) Tenny- parker changes



(a) (b)



(c)

Figure 1.3: Representation of microscopic images of mild, severe PIH placenta.

a) Avascular villi b) Villous hyper maturityc) Fibrin deposition

CONCLUSION

Pregnancy-induced hypertension(PIH) adversely affects the health of the foetus through its harmful effects on the placentas.The observations from the present study could find that there is a significant difference between the calcifications, RPH, FD,TPC, AV, VHM of normotensive mild and severe PIH pregnancies. These changes compromise utero-placental blood flow and significantly reduce the neonatal birth weight. Thus ,this study helps the clinician for early diagnosis and treatment to lower the mortality rates of mothers and fetuses. Due to the long-term risks of high blood pressure, specific cardiovascular risk factors should be addressed and suitable recommendations must be made addressing dietary and lifestyle modification including exercise and maintenance of ideal body weight by taking the suggestions from gynecologist.

CONFLICT OF INTEREST: NIL

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