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Comparison of gross morphological and histological features of placenta between hypertensive and normotensive pregnant women attending Muhimbili National Hospital.

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Abstract

Background: Placenta is the vital fetomaternal organ that is responsible for the maintenance of the pregnancy and promotion of fetal growth and development. The optimal survival, growth, and development of the fetus corresponds to the appropriate formation and development of the placenta. Hypertensive disorders of pregnancy exert a great impact on the placenta and reflect changes both morphological and histological.

Objective: To compare the morphological and histological features of the placenta between hypertensive pregnant mothers and non-hypertensive pregnant mothers who attended Muhimbili National Hospital.

Methods: A hospital-based cross-sectional study was conducted from June 2020 to August 2020 at Muhimbili National Hospital. A total of 80 placentas were studied morphologically and histologically, of which 40 placentas were from hypertensive pregnant mothers and 40 placentas were from normotensive pregnant mothers. In the morphological aspect shape, weight, the site of insertion of the umbilical cord, the number of cotyledons of each placenta and thickness of the placenta were noted. In the histological part, Neutral buffered formalin (NBF) was used the routine staining technique which was Hematoxylin and Eosin (H&E) and a light microscope was used. The data were analyzed by using SPSS version 20 computer software and the results were summarized in means and proportions. 'T-test' and chi-square' tests were used and the difference was statistically significant when the p-value>0.05

Results: The irregular shape of the placenta and marginal insertion of the umbilical cord were more in the hypertensive group and were statistically significant (p>0.01). The mean placenta weight, mean placenta thickness and mean numbers of cotyledons were significantly less in a hypertensive group compared to the normotensive group (p<0.01). A microscopic study of the placenta revealed the presence of fibrinoid necrosis, syntial knots, calcifications and villous hypoplasia in both normotensive and hypertensive groups however these findings were significantly higher in the hypertensive group (p>0.01).

Conclusion: Hypertensive disorders of pregnancy (HDP) exert a profound impact on the placenta. Morphologically altering its dimensions and histologically fibrinoid necrosis, calcifications, syntial knots, and villous hypoplasia were significantly more in the hypertensive group than in the normotensive (p>0.01).

Keywords: placenta, morphology, histology, hypertension

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Introduction

The placenta is the vital fetomaternal organ that is responsible for the maintenance of the pregnancy and promotion of fetal growth and development (Communication, 2017). It connects the fetus to the uterine wall thus providing nutrients and oxygen to the fetus, eliminating waste products from the fetus; working as the digestive system, lung and kidney respectively (Pazinato *et al.*, 2016). The optimal survival, growth and development of the fetus are correspondence to the appropriate formation and development of the placenta (Wang and Zhao, 2010). It develops after conception at the time of implantation of the blastocyst in the uterus, and is generally discharged from the uterus following infant birth. It develops from the fetal tissue which is the chorion frondosum and maternal tissue which is decidua basalis. The normal "term placenta" is round and flat in shape and is about (400 – 600) gm in weight, (15 – 25) cm in diameter, (2 - 3) cm in thickness and 15 to 20 cotyledons however there is a considerable variation from placenta to placenta (Huppertz, 2008).

The histology of the normal placenta, the chorionic surface shows cuboidal to columnar cells in the areolar region and the clumps of chorionic villi (Avagliano *et al.*, 2016). Hypertensive disorders of pregnancy are common and refer to a cluster of disease ranging from chronic hypertension and gestational hypertension to pre-eclampsia –eclampsia (Shah and Gupta, 2019). They are associated with an increased risk of adverse outcomes to both mother and fetus and the common ones are; placenta abruption, intrauterine fetal growth restriction (IFGR), premature delivery, and stillbirth, thus, forming one of the deadly triads along with hemorrhage and infection (Singh and Singh, 2015).

Hypertensive disorders of pregnancy exert a great impact on the placenta. The impact of the hypertensive disorders of pregnancy on the placenta reflects changes both morphological and histological (Heazell, 2015). Previous morphological studies of the placentas revealed that the mean weight and mean a number of cotyledons were significantly less in the hypertensive pregnant mothers compared to normotensive pregnant mothers. Also altered shapes of the placentas that are in irregular shape and marginal insertion of umbilical cord were significantly more in the hypertensive group as compared to the normotensive group (Goswami and Shah, 2016) (Sankar *et al.*, 2013) (Chhatwal, Chaudhary and Chauhan, 2018) (Communication, 2017) (Nag and Vk, 2013) though the study done by (Ashfaq, Janjua and Channa, 2005) showed that the placentas were roughly oval with the central attachment of umbilical cord in both groups.

Previous histological studies of the placentas have revealed the presence of calcifications, infection, fibrinoid necrosis, syntial knots, villous hyalinization in both groups however these findings were significantly predominant in the hypertensive group (Parmar, Shah and Alamchandani, 2019) (Salmani *et al.*, 2014) (Nag and Vk, 2013) (Sankar *et al.*, 2013) (Chhatwal, Chaudhary and Chauhan, 2018). These studies have been done among Caucasians and have revealed some different findings in some aspects. None of the studies was done in our setting, thus we did our study to reveal if these differences existed in our population. Thus, we did in order to compare he morphological and histological features of placenta between hypertensive pregnant mothers and non-hypertensive pregnant mothers who attended Muhimbili National Hospital (MNH).

Materials and Methods

It was a cross-sectional survey that was conducted at Muhimbili National Hospital (MNH), a tertiary hospital in Dar-as-salaam, Tanzania during the period from June 2020 to August 2020. Subjects were recruited from the Obstetrics and labor wards after written informed consent was obtained. We recruited 80 pregnant women with a gestational age of 34 to 42 weeks by the convenient sampling method and this sample size was calculated based on a study by (Noubiap *et al.*, 2019) which aimed in reviewing the burden of hypertensive pregnancy disease in Africa and documented in Tanzania to be 15%.

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Out of the 80 pregnant women included in our study, 40 were normotensive pregnant mothers defined as having persistent blood pressure of < 140/90 mmHg and 40 were hypertensive pregnant mothers who were diagnosed to have Hypertensive Disorder of Pregnancy (HDP) and this was defined as having persistent blood pressure of >140/90 mmHg. Women with multiple pregnancies, diabetes mellitus, hypothyroidism, positive VDRL test and severe anemia (hemoglobin < 6gm/dl) were excluded from the study. Age, gravid status and gestational age at enrolment were noted. Assessment of gestational age was done from the last menstrual period and for those who didn't remember was taken from the ultrasound results. During enrolment, weight and blood pressure of the subjects were noted. All the women were followed up till delivery. After delivery placenta with attached membranes and the umbilical cord was collected, washed in running water, labelled and then fixed with 10% formalin for 2 weeks. Then a gross and microscopic examination of the placenta was carried out. The shape was examined by inspection and was categorized as circular (oval) or irregular.

Site of insertion of the umbilical cord: by inspection and was categorized as central or marginal. The number of cotyledons: by inspection and counting. Thickness: with a long-calibrated needle, the placental thickness was measured at the center in centimeters. Weight: measured by weighing machine in grams. Then 2 cm wedge placental tissue was taken for routine hematoxylin and eosin was made for the histological studies. Sections were examined and photographed using a light microscope (Leica® DM 750) with an in-built camera ((Icc50 HD-47142065) and the number of Islet cells was measured in 40 high-power fields.

Statistical Package of Social Sciences (SPSS) computer software version 20 was used to analyze the data. Descriptive statistics such as proportions for categorical variables and means for continuous variables were estimated. Statistical significance of difference between two groups in the aspect of the data that was expressed in ' means' such as weight of placenta, thickness of placenta, number of cotyledons, number of infarctions, number of syntial knots was calculated by using Students "t" test. A difference between the two groups was considered to be significant when $p<0.05$. In the aspect of the data that was expressed in percentage such as shape of the placenta, site of insertion of umbilical cord statistical significance of difference between two groups was calculated by using chi-square test. A difference between two groups was considered to be significant when $p<0.05$.

Ethical approval

It was approved by Muhimbili University of Health and Allied Sciences research and publication committee and permission to conduct the study was granted by the authority of Muhimbili National Hospital.

Results

In this study, 80 placentae; 40 from normotensive and 40 from hypertensive mothers were used. There was predominance of prim gravida in the hypertensive group that is 67.5% and this difference was statistically significant (p=0.007). In the hypertensive group, 72.5% subjects delivered at pre term in contrast to the normotensive group, 77.5% females delivered at full term. Statistically, a significantly higher number of females delivered preterm in hypertensive group (0.000) (table 1). The irregular shape of the placenta was more predominant in the hypertensive group and it was statistically significant. (p=0.001) The marginal insertion of the umbilical cord was more in hypertensive group and it was statistically significant (p=0.000)The mean placenta weight, mean placenta thickness and mean number of cotyledons were significantly less in the hypertensive group compared to normotensive group. (p=0.000) (table 2). Microscopic study of the placenta revealed the presence of fibrinoid necrosis, syntial knots, calcifications and villous hypoplasia in both normotensive and hypertensive group, however these findings were significantly higher in the hypertensive group than in the normotensive group. (P >0.01) (table 3 and figure 1).

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Table1. Maternal characteristics between hypertensive and normotensive group

Study parameter	Normotensive group		Hypertensive group	P value
Mean weight of pregnant women (kg)	64.33±7.97		66.08±7.03	0.3
Mean systolic blood pressure (mmhg)	121.00±10.04		157.65±10.82	0.000
Mean diastolic blood pressure (mmhg)	76.33±8.45		106.23±8.23	0.000
	N (%)		N (%)	
Age of pregnant woman in years	<20	2(5%)	4(10%)	0.423
	20 – 30	28(70%)	22(55%)	
	>30	10(25%)	14(35%)	
Gravid status of pregnant woman	prim gravid	14(35%)	27(67.5%)	0.007
	Multigravida	26(65%)	13(32.5%)	
Gestational age at delivery in weeks	Preterm*	9(22.5%)	29(72.5%)	0.000
	Full term*	31(77.5%)	11(27.5%)	

Preterm*: Delivery at gestational age between 34 and 36 weeks
Full term*: Delivery at gestational age from 37 to 42 weeks

Table2. Gross morphological features of placenta between hypertensive and normotensive group

Study parameter	Normotensive group		Hypertensive group	p-value
Mean weight of placenta(kg)	548.13±44.33		398.28±71.1	0.000
Mean thickness of placenta(cm)	3.10±0.15		2.28±0.25	0.000
Mean number of cotyledons	18.50±1.20		16.10±0.87	0.000
	N (%)		N (%)	
Shape of placenta	Ovoid	31(77.5%)	15(37.5%)	0.001
	Not ovoid	9(22.5%)	25(62.5%)	
Site of insertion of umbilical cord	Central	34(85%)	17(42.5%)	0.000
	Not central	6(15%)	23(57.5%)	

Table3. Comparison of placental histological features in hypertensive and normotensive group

Parameter	Presence/absence	Normotensive group N (%)	Hypertensive group N (%)	P value
Fibrinoid necrosis	Present	19(47.5%)	32(80.0%)	0.005
	Absent	21(52.5%)	8(20.0%)	
Syntial knots	Present	24(60.0%)	36(90.0%)	0.004
	Absent	16(40.0%)	4(10.0%)	
Calcifications	Present	9(22.5%)	27(67.5%)	0.000
	Absent	31(77.5%)	13(32.5%)	
Villous hypoplasia	Present	8(20.0%)	26(65.0%)	0.000
	Absent	32(80.0%)	14(35.0%)	

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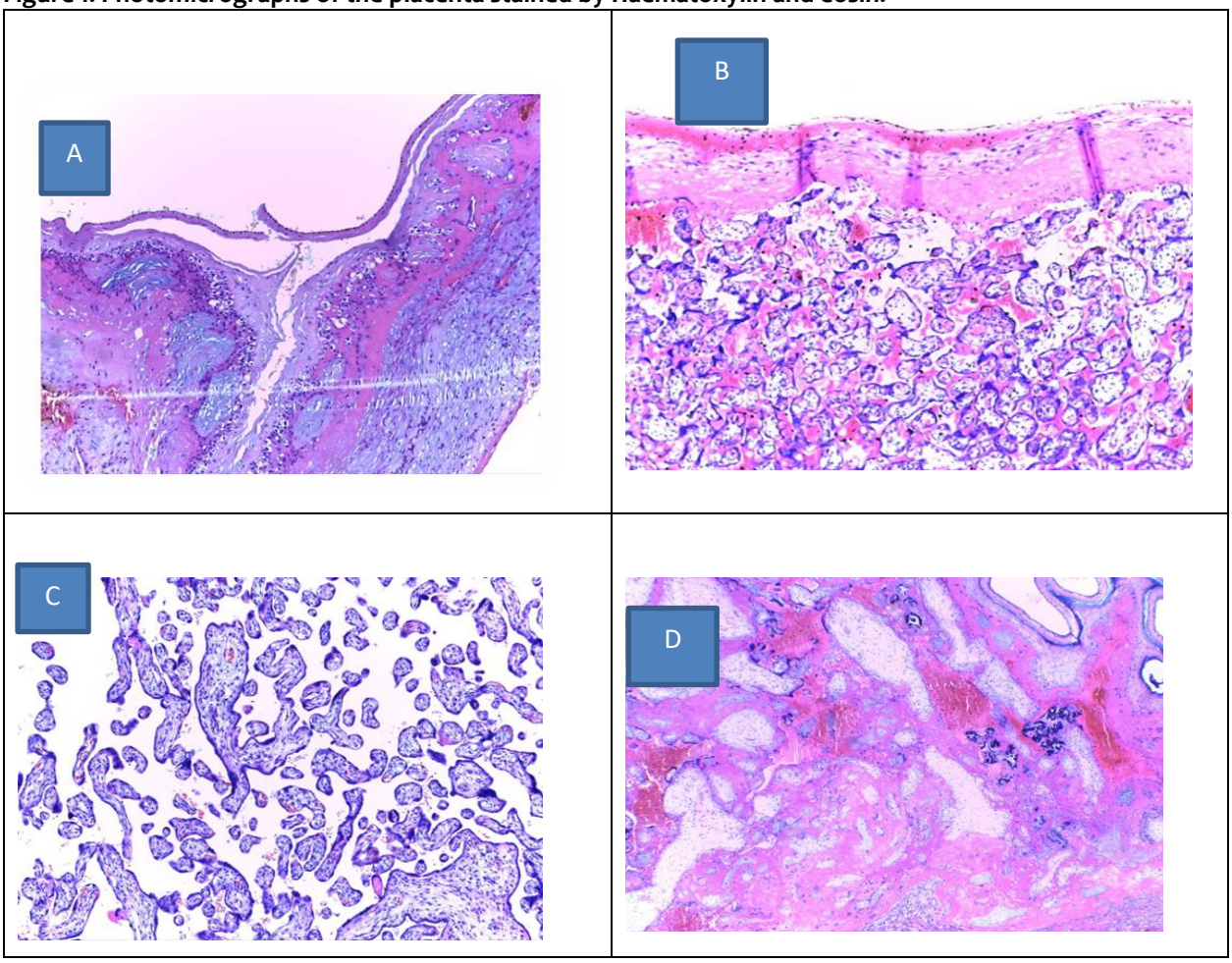
Figure 1: Photomicrographs of the placenta stained by Haematoxylin and eosin.



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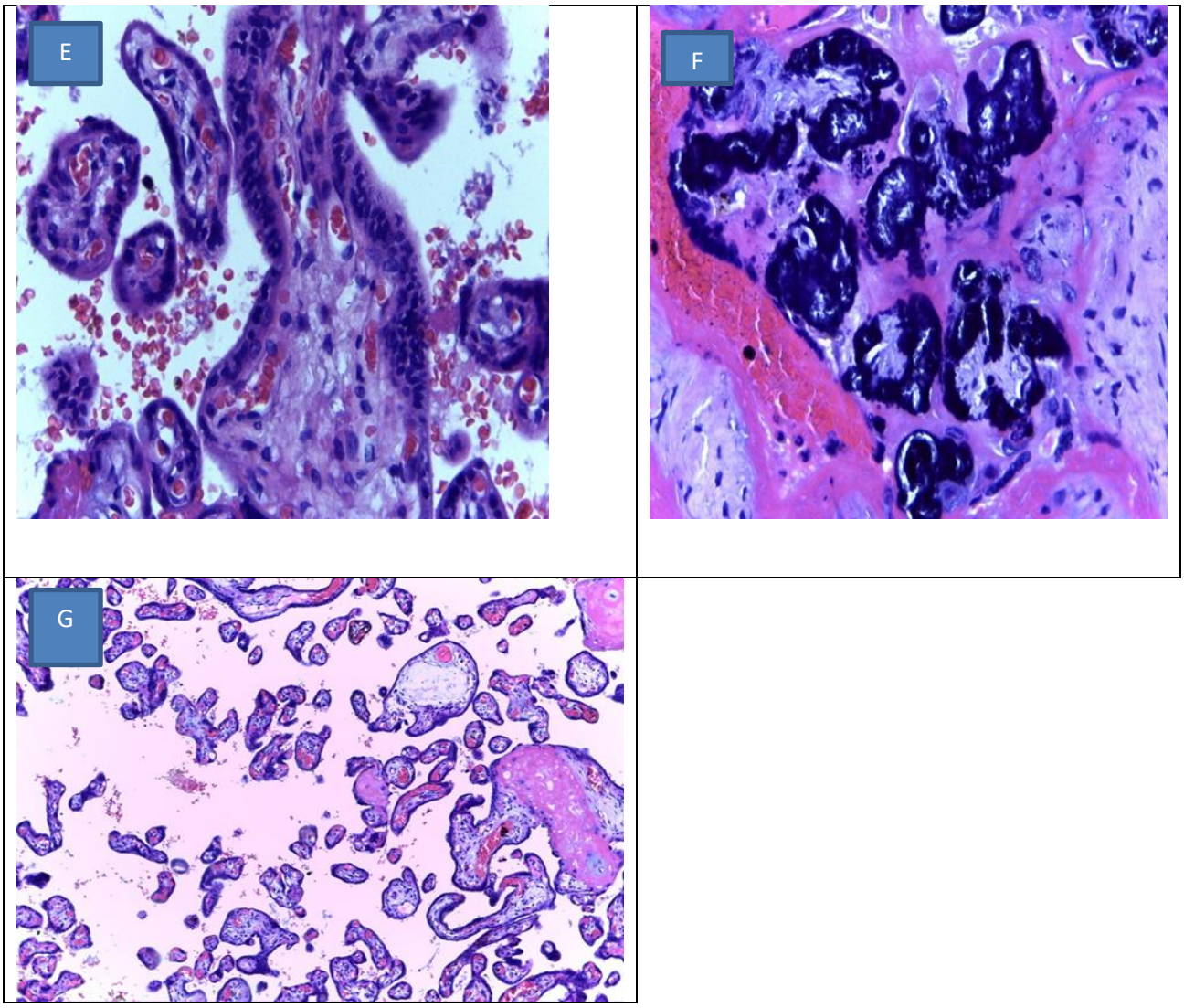

A: Fetal surface of the placenta x 10. It is made of single layered epithelium, the amniotic cells. **B:** Maternal surface of the human placenta x10 magnification. It is incised by placental septa forming cotyledons.
C: Normal chorionic villi micrographic appearance.
D: Fibrinoid necrosis x 4. There is deposition of fibrin surrounding the crowded chorionic villi.
E: Syntial knots x 40. These are recognized as aggregates of snyntiotrophoblastic nuclei.
F: Placental calcification x40.
G: Villous hypoplasia, smaller and slender villi with wide intervillous space.

Discussion

In our study we compared the placental gross morphological and histological features between hypertensive and normotensive pregnant women attending Muhimbili National Hospital (MNH). There was predominance of prime gravida in the hypertensive group and was statistically significant. Prime gravida is one of the etiological factors of hypertensive disorders of pregnancy and this was confirmed in our study since the majority of mothers with hypertensive disorder of pregnancy were prim gravida. This finding is also confirmed by other previous studies (Kambale *et al.*, 2016, Parmar, Shah and Alamchandani, 2019).

Hypertensive group had significantly high blood pressure as compared to normotensive group. Parmar *et al,* 2019 has reported significantly high blood pressure in the hypertensive group as compared to normotensive just as it was observed in our study (Parmar, Shah and Alamchandani, 2019) The current study revealed that preterm deliveries were significantly high in the hypertensive group as compared to normotensive group. This might be attributed to the vulnerability of mothers with hypertensive disorders of pregnancy to premature delivery which is in some circumstances the pregnant woman should be delivered irrespective of gestation age. This finding is as it was ever observed in other studies done earlier (Parmar, Shah and Alamchandani, 2019).

The placental weight is functionally important parameter as it reflects the villous area and fetal metabolism (Communication, 2017). This study revealed that the mean placental weight was significantly less in hypertensive group as compared to normotensive group. These observations correlate well with the previous studies done by various workers (Sankar *et al.*, 2013, Nag and Vk, 2013, Goswami and Shah, 2016, Ashfaq, Janjua and Channa, 2005, Salmani *et al.*, 2014, Kambale *et al.*, 2016, Kartha, Poothiode and P S, 2014). These findings may be attributed to marked reduction in the blood flow in the maternal intervillous space. Though the placenta adapts well to the hypoxic condition in the hypertensive disorders of pregnancy, these compensatory changes that occur are insufficient. These compensatory changes cause underdevelopment and inadequate placental mass (Sankar *et al.*, 2013).

The results from this study revealed that the mean placental weight was more decreased as compared to the previous studies and this may be attributed by genetic and geographical differences. In our present study mean placenta thickness and mean number of cotyledons were significantly decreased in the hypertensive group as compared to normotensive group. These findings were in concordance with previous studies (Communication, 2017, Chhatwal, Chaudhary and Chauhan, 2018, Goswami and Shah, 2016, Communication, 2017). These findings may be attributed by marked reduction in the blood flow in the maternal intervillous space which eventually causes underdevelopment and inadequate placental mass. In our study the irregular shape of the placenta and marginal insertion of umbilical cord were statistically more in hypertensive group. Goswami and Shan have reported the same finding (Goswami and Shah, 2016), however in other studies it was revealed that the placenta was roughly oval with central attachment of umbilical cord in all groups and this finding may be attributed by small sample size that is 20 placenta from hypertensive group and 20 from normotensive group (Ashfaq, Janjua and Channa, 2005).

Microscopic examination of the placenta revealed the presence of fibrinoid necrosis, syntial knots, calcifications and villous hypoplasia in both hypertensive group and normotensive group however they were significantly more in the hypertensive group. Fibrinoid necrosis was seen in 80% and 47.5% in hypertensive and normotensive group respectively. Calcifications noted in 67.5% and 22.5% in hypertensive and normotensive groups respectively. Villous hypoplasia was noted in 65% and 20% in hypertensive and normotensive group respectively.

A significant increase in fibrinoid necrosis, infarction and villous hypoplasia in placental tissue in the hypertensive group indicates the disturbance in the blood flow. Reduction in blood flow is due to vasculopathies of the spiral arteries, that is maternal vasospasm. These findings were similar to findings of previous studies (Salmani *et al.*, 2014, Tateishi *et al.*, 2018, Goswami and Shah, 2016, Sankar *et al.*, 2013, Kartha, Poothiode and P S, 2014). Syntial knots were noted in 90% and 60% in hypertensive group and normotensive group respectively. This finding was significantly higher in hypertensive group as compared to normotensive group. This finding was in line with the observations revealed by other authors (Parmar, Shah and Alamchandani, 2019, Salmani *et al.*, 2014). This observation may be attributed by reduced blood flow through villi results in stromal fibrosis and thus excess syntial knot formation and thus reflecting placental hypoxia/ischemia

Conclusion

Prim gravida females were among the commonly affected group by hypertensive disorders of pregnancy which exert a profound impact on the placenta. Significant changes in gross morphology have been observed more the in hypertensive group. Reduction of weight, numbers of cotyledons and altering its dimensions including the shape the of placenta and site of insertion of umbilical cord have been observed more significantly in the hypertensive group. They induce histological changes including fibrinoid necrosis, calcifications, syntial knots, and villous hypoplasia. Thus, pregnant women with pregnancy disorders of pregnancy have an increased chance of ischemic damage to the placental tissue along with mal-developed terminal villi.

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