Original
ISSN 2521-8492

Study of placental shape and histopathological changes in pregnant ladies with pre-eclampsia

Ban Amer Mousa[1], Sijal Fadhil Farhood Makki Al Joborae[2]

[1] Department of Gynecology and Obstetrics, Collage of Medicine, University of Babylon, Babylon, Iraq.
[2] Department of Community Medicine, Collage of Medicine, University of Babylon, Babylon, Iraq. sijalaljoborae@yahoo.com
Correspondence to Ban Amer Mousa (E-mail: Ban200372@gmail.com)
(Submitted: 08 September 2018 – Revised version received: 12 October 2018 – Accepted: 19 October 2018 – Published online: 26 June 2019)

Objectives To discover the morbid changes (macroscopic and microscopic) in placenta in cases of pre-eclampsia and to correlate the findings with birth weight and outcome of newborn babies in comparison with normotensive mothers.
Methods A case–control study carried out in Babylon teaching for maternity and children hospital, in a period of 12 months from Jan. 2017 to the end of Dec. 2017. One hundred twenty pregnant women were enrolled in the current study consisting of 60 normotensive pregnant women and 60 patients with pre-eclampsia. The placenta of the participants was studied (morphologically and histopathologically), fetal outcome whether alive or dead was recorded and neonatal birth weight was assessed.
Results In the present study, it was observed that weight and dimensions of placenta were less in study group when compared with control group while retroplacental clots are more significantly common in study group than control group. The mean neonatal birth weight was more in normal pregnant ladies and also fetal outcome is significantly better in normotensive group due to normal placental conditions. Histopathological study showed significant number of syncytial knots, trophoblastic basement membrane thickness, cytotrophoblastic cell proliferation, areas of fibrinoid necrosis, hyalinization, calcification, and areas of infarction.
Conclusion Pre-eclampsia has effects on the patient's placenta by decreasing its dimension and weight in addition to presence of retroplacental clots that is due to several histopathological changes that affect the normal functions of it which lead to reduce the neonatal birth weight and may result in intrauterine death. Hence, we must screen about this disease as early as possible to discover and manage it earlier and decrease its complications in high risk pregnancies.
Keywords Pregnancy, placenta, pre-eclampsia, morbidity, mortality

Introduction

The placenta is an organ that connects the growing fetus to the uterus to allow nutrient supplements, waste products elimination, and gas exchange through the mother's circulation. Normal fetal development and survival depends on the intact function of the placenta. In normal pregnancies, the wall of the spiral arteries is invaded by trophoblastic cells and transformed into large, tortuous channels that carry a large amount of blood to the intervillous space and are resistant to the effects of vasomotor agents. Trophoblastic invasion begins by causing destruction of the muscularis layer of spiral arteries land and is completed by 24 weeks.[1] Accommodations of the maternal uterine spiral arteries by the invasion through trophoblast and increased perfusion of the intervillous placental spaces are necessary for the normal development of pregnancy. In spite of the multiple theories for its pathophysiology, it is commonly accepted that pre-eclampsia (PE) is certainly associated with defective trophoblast invasion and failure of the normal physiological adaptations. This results in fetal hypoxia and/or stress and the typical villous and vascular placental lesions associated with PE.[2-4] The main feature of abnormal placentation is inadequate trophoblastic invasion of the maternal spiral arteries. This results in persistence of muscular and elastic tissues of the media of spiral arteries. As a result, the vessels fail to dilate and remain responsive to vasomotor effects, which lead to high resistance and low flow choriodecidual circulation. With progress of pregnancy, the metabolic demands for intact fetoplacental unit elevated but the spiral arteries are unable to dilate to accommodate the required elevated in blood flow, resulting in placental insufficiency.[5]

Microscopic (histologic) description

1 Increased syncytial knots,
2 thickening of trophoblastic basement membrane,
3 cytotrophoblastic cell proliferation,
4 infarction,
5 fibrinoid necrosis,
6 hyalinization of uterine vessels.
7 acute atherosis; more tortuous or densely distributed spiral and basal arteries than normal that lead to inappropriate trophoblastic maturity.[7]

During fetal development throughout the pregnancy, placenta undergoes a lot of changes in weight, length, and function. When pregnancy is complicated by a PE, it affects the functions of placenta. Thus, examination of placenta gives good information about the expected perinatal health of the baby and mother.

Hypertension in pregnancy is divided into chronic hypertension (onset before pregnancy), pregnancy-induced hypertension (PIH), PE and eclampsia (complicated form). It is one of the deadly triad in addition to bleeding and infection resulting in maternal and fetal mortality.[8]

Materials and methods

This study was carried out in the Department of Obstetrics and Gynecology at Babylon teaching hospital. The study was done on 120 placentae, which were collected from the labor ward and operating room, 60 placentae from uncomplicated full-term pregnancies (the control group) and the others from PE patients after taking consent from them.

Exclusion criteria

1 Cases with hypertension prior to current pregnancy, PIH, hypertension secondary to other chronic causes such as associated with cardiac diseases, renal disorder and diabetes mellitus, etc.
2 Anemia.
3 Multiple gestations.
4 Pregnancy ≤ 37 weeks of gestation.
5 Post-dated pregnancies.
6 Placenta praevia which indicate abnormalities of placental insertion.

After admission of the patient to the hospital, history, examination and fetal wellbeing were assessed whether alive fetus or dead. Some investigations were done for her to assess maternal wellbeing and after delivery weight of newborn baby were recorded. All the placentae with attached membrane and umbilical cord were collected soon after delivery, followed by the presence of retroplacental clots and visible calcification will checked immediately by naked eyes. Then washed it in running tap water, to clean all the blood and after that fixed in 10% formalin. The length and the weight of the placenta were measured. Multiple pieces of the placenta were taken wherever microscopic lesions were suspected. Tissues were stained with a special stain and send to the laboratory to confirm the villous pathology. Histopathological study of tissue samples was done and the slide were studied under the light microscope.

Evaluation of histological features such as syncytial knots, trophoblastic basement membrane thickness, cytotrophoblastic cell proliferation, areas of fibrinoid necrosis, hyalinization, calcification, and areas of infarction was done.

All the gross and histological features were compared between placentae obtained from PE pregnancies and others from uncomplicated pregnancies (control group).

Results

Fig. 1 shows that 35% of the hypertensive women had mild degree of hypertension while 55% presented with severe degree and the last 10% eclampsia.

Figure 2 shows that the mean age of women in the case group was 27.22±8.25.

Table 1 shows that the mean gestational age of the neonates was 38.35±1.02, 65% of hypertensive women were multigravida and 28.3% born via Caesarian section, regarding outcome of the neonate 16.7% of them were stillbirths.

Table 2 shows that *t*-test was conducted to find the mean difference of placental weight, and length (macroscopic gross features) according to study groups. There was a significant mean differences in both circumstances (*p*-value less than 0.001).

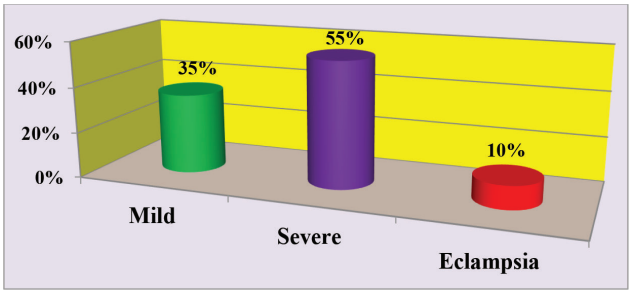

Fig. 1 **Distribution of hypertensive patients according to severity.**

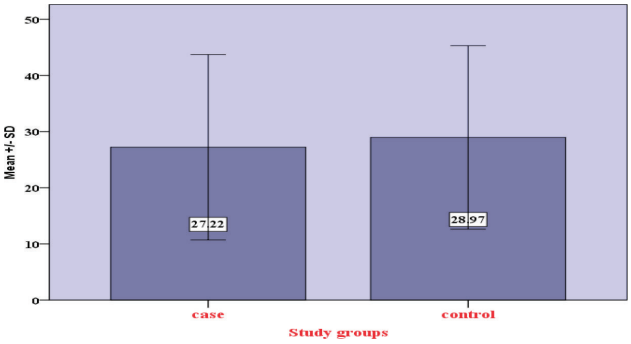

Fig. 2 **Mean age of study groups.**

Table 1. **Distribution of variables in cases group.**

Variables	Mean ± SD	Range
Gestational age (wk.)	**38.35±1.02**	**(37-40)**
Outcome of neonate		
Alive	50	83.3%
Dead	10	16.7%
Total	60	100.0%
Gravida		
Primi gravida	21	35.0%
Multi gravida	39	65.0%
Total	60	100.0%
Mode of delivery		
Caesarian section	17	28.3%
Vaginal delivery	43	71.7%
Total	60	100.0%

Table 3 shows that chi-square test was conducted to find the association between retroplacental clot (macroscopic gross feature) according to study groups. There was a significant association with *p*-value less than 0.001

Table 4 shows that chi square test was conducted to find the association between placental microscopic features and study groups.

There were significant associations in all circumstances (*p*-value ≤.0.05).

Table 5 shows that *F*-test was conducted to find the mean differences of maternal age, birth weights of neonate according to severity of maternal hypertension in cases group. There was a significant mean difference of birth weight of neonates according to severity of maternal hypertension (*p*-value<0.001) (Table 6). There was no significant mean difference of maternal age according to severity of maternal hypertension (*p*-value >0.001)

Table 7 shows that *t*-test was conducted to find mean differences of placental weight and length (macroscopic gross features) according to the outcome of neonates in cases group. There was a significant mean difference in both circumstances (*p*-value 0.002 and <0.001 respectively)

Table 8 shows that Fisher exact test was conducted to find an association between retroplacental clot (macroscopic gross features) and outcome of neonates in cases group. There was a significant association (*p*-value < 0.001).

Table 2. **The mean differences of macroscopic gross features of placenta according to study groups.**

Variable	Groups	N	Mean ± SD	*t*-test	*p*-value
Placental weight (gm)	Case(<400) Control(400)	60 60	395.5±50.03 439.17±19.42	-6.302	<0.001*
Placental length (cm)	Case(<17) Control(>17)	60 60	16.83±1.74 18.65±1.05	-6.892	<0.001*

*p-value≤ 0.05 was significant.

Table 3. **Association between gross features of placenta and study groups.**

Variables	Study groups		Total	X^2 test	*p*-value
	Case	Control			
Retroplacental clots					
Present	24(40.0%)	2(3.3%)	26(21.7%)	23.764	<0.001*
Absent	36(60.0%)	58(96.7%)	94(78.3%)		
Total	60(100.0%)	60(100.0%)	120(100.0)		

*p-value≤ 0.05 was significant.

Table 4. **Association between placental microscopic features and study groups.**

Variables	Study groups		Total	X^2 test	*p*-value
	Case	Control			
Syncytial knots					
Present	17(28.3%)	3(5.0%)	20(16.7%)	11.76	0.001*
Absent	43(71.7%)	57(95.0%)	100(83.3%)		
Total	60(100.0%)	60(100.0%)	120(100.0)		
Cytotrophoblastic cell proliferation					
Present	16(26.7%)	5(8.3%)	21(17.5%)	6.984	0.008*
Absent	44(73.3%)	55(91.7%)	99(82.5%)		
Total	60(100.0%)	60(100.0%)	120(100.0)		
Trophoblastic basement membrane thickening					
Present	31(51.7%)	3(5.0%)	34(28.3%)	32.15	<0.001*
Absent	29(48.3%)	57(95.0%)	86(71.7%)		
Total	60(100.0%)	60(100.0%)	120(100.0)		
Fibrinoid necrosis					
Present	24(40.0%)	2(3.3%)	26(21.7%)	23.764	<0.001*
Absent	36(60.0%)	58(96.7%)	94(78.3%)		
Total	60(100.0%)	60(100.0%)	120(100.0%)		
Calcification					
Present	21(35.0%)	2(3.3%)	23(19.2%)	19.417	<0.001*
Absent	39(65.0%)	58(96.7%)	97(80.8%)		
Total	60(100.0%)	60(100.0%)	120(100.0%)		
Hyalinization					
Present	34(56.7%)	2(3.3%)	36(30.0%)	40.635	<0.001*
Absent	26(43.3%)	58(96.7%)	84(70.0%)		
Total	60(100.0%)	60(100.0%)	120(100.0%)		
Infarction				19.806	<0.001*
Present	17(28.3%)	0(0.0%)	17(14.2%)		
Absent	43(71.7%)	60(100.0%)	103(85.8%)		
Total	60(100.0%)	60(100.0%)	120(100.0%)		

*p-value≤ 0.05 was significant.

Discussion

Placenta is a fetal organ that sustains the same strain and stress, which the fetus bares. It forms the record of anatomical circumstances, intrauterine, and intrapartum events of the pregnancy.[9] Pregnancies complicated by PE are revealed in the placenta both macroscopically and microscopically. Although the placenta adapts well to the hypoxic circumstances in PE, the compensatory alterations that happened are insufficient. These compensatory alterations cause maldevelopment and insufficient placental mass, causing placental dysfunction that leads to oxidative stress and chronic fetal hypoxemia.[10] In

Table 5. **The mean differences of maternal age, birth weight according to severity of hypertension in patients group.**

Variable	Groups	N	Mean ± SD	*F*-test	*p*-value
Age (years)	Mild	21	28.95±7.37	2.06	0.137
	Severe	33	27.18±8.33		
	Eclampsia	6	21.33±9.39		
	Total	60			
Birth weight (gm.)	Mild	21	3228.57±498.13	19.01	<0.001*
	Severe	33	2621.21±508.52		
	Eclampsia	6	1850±644.2		
		60			

*p-value≤ 0.05 was significant.

Table 6. **The mean differences of birth weight according to study groups.**

Variable	Groups	N	Mean ± SD	*t*-test	*p*- value
Birth weight (gm)	Case	60	2756.67±657.77	**-2.237**	**0.027***
	Control	60	2995±498.61		
	Total	120			

*p-Value≤ 0.05 was significant.

Table 7. **Mean differences of macroscopic gross features of placenta according to neonatal outcome in patients group.**

Variable	Fetal outcome	N	Mean ± SD	*t*-test	*p*-value
Placental weight (gm)	Alive	50	402.2±50.96	3.536	0.002*
	Dead	10	362±27.8		
	Total	60			
Placental length (cm) (longitudinal diameter)	Alive	50	17.2±1.59	4.088	<0.001*
	Dead	10	15±1.33		
	Total	60			

*p-value ≤ 0.05 was significant.

Table 8. **Association between macroscopic gross features of placenta and neonatal fate in patients group.**

Variables	Fate		Total	*p*-value
	Alive	Dead		
Retroplacental clots				
Present	15(30.0%)	9(90.0%)	24(40.0%)	0.001*
Absent	35(70.0%)	1(10.0%)	36(60.0%)	
Total	50(100.0%)	10(100.0%)	60(100.0%)	

*p-value≤ 0.05 was significant.

PE, placenta has a tendency to be smaller as compared with uncomplicated gestation.[5] The flow of the blood to placenta is reduced in hypertension of pregnancy and result in improperly small fetus. Also hypoxia may lead to reduced placenta weight. In the present study, comparing PE placentae with control placentae, the mean placental weight and length were declined significantly. The placental weight is an important cause for both fetal growth and birth weight. It is functionally important as it is related to villous area and fetal metabolic rate.[11] The mean birth weight of the baby in control group was 2995 g, whereas in the PE group was 2756.67 g.

The mean weight of the placenta in the control group was 402.2 g, whereas in the PE group was 362.27 g. Same findings were statistically significant and similar to others studies like Udainia and Jain, and Nag et al studies.[12,13] In our study, the mean longest transverse length of placentae in the control group was 17.2 cm, whereas in the PE group was 15.1cm. In this study, length of placenta (transverse length) in PE group was significantly decreased. Teasdale found significant decrease of transverse length in PE group (and that similar to our study); this reduction appears to be due to the small size of placenta in this group.[14] Cibils said that the placentae from patients with hypertension were significantly smaller than the normal pregnant, proposing that the pathological process has happened and affected the normal placental functions and development.[15] Despite the higher likelihood of finding abnormal placental pathology in pregnancies with PE, these lesions are not specific to the diagnosis of PE (same pathology might be discovered in other diseases). However, as per the reports on the incidence, nature and severity of the placental histopathological abnormalities in PE is conflicting. [16–22] In our study, we found significant difference in placental calcification (35%),(3.3%) and infarction (28.3%),(0%) between hypertensive and normotensive groups, respectively, and it is the same

as with the studies of Motwani et al which found the incidence of calcification and infarction were elevated in hypertensive as compared with normotensive groups[23] and Qureshi et al, in their study found infarction was elevated in hypertensive as compared with the normotensive ladies.[24] Singh and Gugapriya studies found incidence of infarction, hematoma, and calcification and the difference is statistically significant for all the three changes.[25] Also, in our study we found higher prevalence rate in previous three changes, hypertensive more than normotensive groups and this was to be statistically significant for these parameters. Chhatwal et al said that fibrinoid necrosis increased in hypertensive cases and some of the normotensive cases.[26] In other studies too, its prevalence has been reported to range from 50% to 70% in hypertensive women.[23–27] For other important findings such as cytotrophoblastic cellular proliferation (51.2%) which was seen in hypertensive group and to lesser spread (8.3%) in normotensive cases also. The same is supported in studies from Motwani et al, Nag et al, and Porwal et al.[19,26,30] For other important change, like hyalinized areas we had 56.7% in hypertensive cases and 3.3% normotensive cases. Motwani et al, in their study, reported hyalinized areas in 46.66% of hypertensive cases and 13.3% of normotensive cases, which is lower than that reported in hypertensive group while higher in normotensive group in our present study.[29] However, the difference might be due to difference in method of assessment. Porwal et al found results supportive to present study with 83.34% positivity in hypertensive cases as compared with 0% in normotensive cases.[28] Thus, use of this criteria was not only more sensitive but also more specific.[29] In present study, stromal fibrosis was seen in 26.2% of hypertensive cases as compared with 2.4% of normotensive cases. Compared with this, Rana et al reported stromal fibrosis in 40% of hypertensive cases and 15% of normotensive cases.[30] The microscopic lesions were assessed as percentage of placenta in PIH and control group showing more than 30% of syncytial knots, villi with 3% of villi with fibrinoid necrosis, stromal fibrosis and basement membrane thickening more than 3% of villi as supported by most literatures. The mode of formation and function of syncytial knot has been considered as a degenerative process and aging change.[31] The placental villous membrane (syncytioplasm) and the fetal capillaries remain separate but act as a single unit, called vasculosyncytial membrane.[20,22] The two factors responsible for the formation of stromal fibrosis are a normal aging process and a reduced uteroplacental blood flow. Fibrinoid necrosis may be a manifestation of endothelial damage in placenta which may lead to increased coagulation tendencies.[32–34] The basement membrane thickening is the byproduct of cytotrophoblast cell hyperplasia, as the basement membrane protein is secreted by these cells. Hence, cytotrophoblastic proliferation is seen as basement membrane thickening in placental ischemia of the PE group.[34] In the present study, >30% of syncytial knots were seen in, 5% placenta of the control group and 28% placenta of the PE group and similar increasing findings are present study by Jena and Shalini,[34] which is statistically significantly increased.

Conclusion

PE has effects on the patient's placenta by decreasing its dimension and weight in addition to the presence of retroplacental clots, which is due to several histopathological changes that affect the normal functions of it which lead to reduction in the neonatal birth weight and may result in intrauterine death. This complication is wide common in our country and mostly diagnosed in late stage and in complicated form (severe PE and eclampsia).

Recommendations

1 We must screen about this disease as early as possible to discover and manage it earlier and decrease its complications in high risk pregnancies.
2 It is preferable to do a lot of researches about this disease to know the exact cause for it and how prevent it can be prevented as much as possible.

Reference

1. Robertson WB, Brosens I and Dixon HG. The pathological response of the vessels of the placental bed to hypertensive pregnancy. J Pathol Bacteriol 1967;93:581–92.
2. Redman CWG. Pre-eclampsia and the placenta. ***Placenta*** 1991;12:301–308.
3. Roberts JM, Redman CW. Pre-eclampsia: more than pregnancy-induced hypertension. ***Lancet*** 1993;341:1447–1451.
4. Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. Pathophysiology of hypertension during pre-eclampsia linking placental ischemia with endothelial dysfunction. ***Hypertension*** 2001;38:718–722.
5. Norwitch ER, Chaur-Dong HSU, Rapke JT. Acute complication of preeclampsia. Clinical Obstet Gynecol 2002;45(2):308–29.
6. Burton GJ, Tham SW. The formation of vasculo-syncytial membranes in the human placenta. J Dev Physiol 1992;18:43–76. Narasimha A and Vasudeva DS. Spectrum of changes in placenta in toxemia of pregnancy. IJPM 2011;54(1):15-18
7. Starzyk KA, Satafia CM, Pezzullo JC, et al. Quantative differences in arterial morphometric define the placental bed in preeclampsia. Hum Pathol 1997;28:353.
8. Louise C Kenny, Jenny E Myers, et al. Obstetrics by ten teachers. Hypertensive disorders of pregnancy. Preeclampsia. 2018;9:253.
9. Salmani D, Purushothaman S, Somashekara SC et al. Study of structural changes in placenta in pregnancy-induced hypertension. J Nat Sci Biol 2014;5(2):352–355.
10. Myatt L. Role of placenta in preeclampsia. Endocrine. 2002;19:103–111. [pubmed]
11. Manjunatha HK, Kishanprasad HL, Ramaswamy AS, Aravindra P, Prakash H Muddegowda. Study of histomorphological changes in placenta in pregnanacy induced hypertension. Int J Cur Sci Res. 2012;2(1):255–258.
12. Udainia A, Jain ML. Morphological study of placenta in pregnancy induced hypertension with its clinical relevance. J Anat Soc India 2001;50(1):24–27.
13. Nag U, Chakravarthy VK, Rao DR. Morphological changes in placenta of hypertensive pregnant women. IJRRMS 2013;3(2):1–4.
14. Teasdale F. Histomorphometry of the human placenta in preeclampsia associated with severe intrauterine growth retardation. Placenta 1987;8:119–28.
15. Cibils LA. The placenta and newborn infant in hypertensive conditions. Am J Obstet Gynecol. 1974 Jan;118(2):256–70.
16. Ogge G, Chaiworapongsa T, Romero R, Hussein Y, Kusanovic JP, Yeo L, Kim CJ, Hassan SS. Placental lesions associated with maternal underperfusion are more frequent in early-onset than in late-onset preeclampsia. J Perinat Med. 2011;39:641–652.
17. Maloney KF, Heller D, Baergen RN. Types of maternal hypertensive disease and their association with pathologic lesions and clinical factors. Fetal Pediatr Pathol. 2012;31:319–323.

18. Devisme L, Merlot B, Ego A, Houfflin-Debarge V, Deruelle P, Subtil D. A case–control study of placental lesions associated with pre-eclampsia. Int J Gynaecol Obstet. 2013;120:165–168.
19. Vinnars MT, Nasiell J, Ghazi S, Westergren M, Papadogiannakis N. The severity of clinical manifestations in pre-eclampsia correlates with the amount of placental infarction. Acta Obstet Gynecol Scand**.** 2010;**90**:19–25.
20. van der Merwe JL, Hall DR, Wright C, Schubert P, Grové D. Are early and late preeclampsia distinct subclasses of the disease – what does the placenta reveal? Hypertens Pregnancy 2010;29:457–467.
21. Pathak S, Lees CC, Hackett G, Jessop F, Sebire NJ. Frequency and clinical significance of placental histological lesions in an unselected population at or near term. Virchows Arch. 2011;459:565–572.
22. Ruiz-Quiñonez G, Reza-López SA, Chávez-Corral DV, Sánchez-Ramírez B, Leal-Berumen I, Levario-Carrillo M. Placental maturity, hypertensive disorders of pregnancy and birth weight. Hypertens Pregnancy 2014;33:132–144.
23. Motwani R, Sontakke Y, Goyal M. Effects of pregnancy induced hypertension on human placenta. JEMDS. 2013;2(33):6275–82.
24. Qureshi MA, Bhurgri GR, Yousfani GM. Morphological and histological changes in placenta of hypertensive and gestational diabetic women. Med Formum Monthly. 2014;25(10):10–4.
25. Singh S and Gugapriya TS. A cross sectional morphometric study of hypertensive with normal placentae and its correlation with fetal outcome. Int J Anat Res. 2014;2(2):437–42.
26. Chhatwal J et al. Int J Reprod Contracept Obstet Gynecol. 2018 Sep;7(9):3808–3813.
27. Shevade S, Arole V, Bharambe V, Paranjape V. Placental morphology and fetal outcome in preeclampsia and normotensive pregnancies. IOSR-JDMS 2015;14(4):11–15.
28. Porwal V, Jain D, Gupta S, Khandelwal S, Kasliwal N. Spectrum of placental changes in pregnancy induced hypertension. Annal Pathol Lab Med. 2017;4(1):A69–A76.
29. A Comparative Study on Placental Changes in Uncomplicated Pregnancies with Pre-Eclamptic Women JMSCR 2018;06(02):1291–1296.
30. Rana S, Diwan Y, Chauhan RS, Diwan D, Gupta A. Comparative study of histology of placenta in normotensive and hypertensive cases. JMSCR. 2017;5(3):18635–40.
31. Spanos S, Rice S, Karagiannis P, Taylor D, Becker DL, Winston RM, Hardy K. Caspase activity and expression of cell death genes during development of human preimplantation embryos. Reproduction 2002;124(3):353–563.
32. Aplin JD. Developmental cell biology of human villous trophoblast: Current research problems. Int J Dev Biol. 2010; 54(2–3):323–329.
33. Loukeris K, Sela R, Baergen RN. Syncytial knots as a reflection of placental maturity: Reference values for 20 to 40 weeks gestational age. Pediatr Dev Pathol. 2010;13(4):305–309.
34. Jena M, Shalini J. Comparative analysis of the villous abnormalities of placentae in pregnancy induced hypertension with that of normal pregnancy. BJMMR2015;9(9):1–9.