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PLACENTAL HISTO-MORPHOMETRIC CHANGES IN PREECLAMPSIA AND ITS ASSOCIATION WITH BETATROPHIN

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Abstract:
Preeclampsia is one of hypertensive disorders in pregnancy that occurs after the 20th weeks of pregnancy along with proteinuria. It threatens maternal and child health and has worldwide prevalence of about 3–8%. This cross-sectional study included 90 cases among them 45 women were diagnosed with preeclampsia and 45 normal pregnant women was carried out from December 2021 to November 2022 at department of anatomy in collaboration with department of gyneacology and obstetric, Liaquat University of Medical and Health Sciences, Jamshoro after getting ethical approval. Placental histological changes were matched for betatrophin levels. Their age, gravidity, gestational age, socioeconomic status, and mode of delivery were recorded. The mean betatrophin level in control 1.66 ± 0.10 and in pre-eclampsia 1.71± 0.06. Plasma betatrophin levels were found significant in PE than in control groups ($p<0.008$). Placental morphological and morphometric parameters were significant in PE than control group. All gross and microscopic parameters of placenta were altered in preeclampsia along with plasma elevated levels of betatrophin. These findings may bring out betatrophin as a new marker to predict, prevent, and treat pre-eclampsia.

Keywords: Betatrophin; Placenta; Pre-eclampsia; Pregnancy Induced Hypertension

INTRODUCTION:

The pregnancy induced, hypertensive disorders are the second most common cause of morbidity and mortality in women worldwide.(1) These disorders complicate pregnancy and result poor maternal and

prenatal outcome affecting 5 to 10% total normal pregnancies worldwide.[2] Pre-eclampsia (PE) is the newly induced hypertension after 20 weeks of pregnancy including proteinuria, dysfunction of maternal organs and restriction of fetal growth. It affects 3%–5% of pregnant women globally. It also occurs after pregnancy in the postpartum period that is referred as postpartum PE.[3] Postpartum PE is an unusual type of PE, arises after delivery presenting with same clinical presentation. PE or eclampsia complicated pregnancies should be monitored intensely and immediately in hospital for treatment with magnesium sulfate via intravenous route. Magnesium sulfate should be used as an antihypertensive therapy.[4, 5] Several factors like; advancing maternal age, past history of PE, nulli- or multiparity, short and long inter-pregnancy intervals, Exposure of hyper-estrogenic ovarian stimulation medications , Medical conditions like preexisting type 1 and type 2 diabetes mellitus, renal disease, chronic hypertension, anti-phospholipid syndrome (APS), autoimmune ailments such as systemic lupus erythematous (SLE) and previous vascular diseases etc. are involved in PE during pregnancy.[6]

PE leads to the development of pathological changes that commonly affects various systems of mother. It affects neurologic, renal, cardiovascular, hematologic, and hepatic systems of the mother along with the adverse effects on the utero-placental unit, causing fetal and neonatal complications as well. The risk of stroke, epilepsy, vascular dementia, and cognitive impairment after pregnancy also increased in women with previous history of PE.[7]

Placenta is the feto-maternal organ that is essential for pregnancy in regulating the growth and protection of the fetus during whole embryonic period.[8] It is the main pathological focal point for PE since the disease is resolved within 24-48 hours after delivery of the placenta. PE affects the uterine spiral arteries of proximal decidua of the placental bed reducing their average external diameter. This vascular remodeling impacts fetal demands to meet increased response as gestation progresses.[9] Genetic predisposition also causing PE as a single recessive gene in the mother that is related to PE. With increasing gestational age, the placenta becomes further ischemic which also leads to PE. Vasoactive agents which bring about changes placental vascularity are Prostaglandins, Endothelins, and vascular Endothelial Growth Factor (VEGF) resulting in PE. PE led to extreme state of activated leucocyte in the maternal circulation, oxidative stress, imbalance between prooxidants and antioxidants causing tissue damage. [3, 10]

PE induces harmful effects on placental morphology include decreased weight, decreased surface area, thickness, oval shape is more common than round shape, feto-Placental index alteration and velamentous insertion.[11] Whereas, under-microscopic evaluation, it results in high number of syncytial knots, Villi hypo-vascularity, Proliferation of cyto-trophoblastic membrane, trophoblastic membrane thickening, fetal capillaries have endothelial cells that are obliterative and enlarged and the placental bed shows spiral arteries atherosis. Moreover, there is thrombotic obstruction of maternal utero-placental vessels that causes placental hematoma and infarcts that further results in calcification.[12]

Beta-trophin (BT) is the angiopoietin-like proteins (ANGPTLs), glycoproteins that are commonly found in tissues like liver, cardiovascular system and haemopoietic system. These proteins engage significantly in inflammation, metabolism of lipids, angiogenesis, apoptosis, metabolism, and cancer progression.[13] During pregnancy due to adipose tissue accumulation, lipolysis, and hyperlipidemia, there is an increased lipid metabolism. The hormones of pregnancy like progesterone and estrogen causes physiological hypercholesterolemia. These increases circulating BT in pregnant women. BT is in relation to estrogen and progesterone that contributes to remarkable BT elevation during pregnancy. BT raises more in 3rd than in the 2nd trimester during pregnancy.[13, 14]

PE is the hypertensive disorder of pregnancy endangering maternal and child health with worldwide prevalence of about 3–8%. Whereas, BT plays significant role in glucose and lipid metabolism. The function of BT is still unclear in PE. Therefore, this study is designed to determine histological changes in the normal and PE women Placentae and its correlation with BT levels.

METHODOLOGY:

The Cross-sectional study was conducted from December 2021 to November 2022 at the Department of Anatomy with collaboration of Department of Gynecology and Obstetrics, Diagnostic and Research laboratory, Liaquat University of Medical and Health Sciences, Jamshoro/Hyderabad after ethical approval. All the pregnant women with or without diagnosed PE were included while all those with previous history of chronic hypertension, known case of diabetes and chronic renal failure were excluded. Sample size of 90 was calculated by Epi-tools Epidemiological online calculator by taking anticipated prevalence 3% [(15)], confidence interval at 95% and margin of error of 5%.

Data collection: Patient's information was gathered soon after their delivery regarding the age, socioeconomic status, gravida, gestational age, and mode of delivery. Whereas, the blood sample of 5ml blood was drawn and collected in the EDTA tubes and were labeled accordingly and centrifugation at 3000 RPM for 15 minutes at 2-8C in Diagnostic and Research laboratory. The obtained serum was Betatrophin levels were analyzed by a Human Angiopoietin-like Protein 8 ELISA kit (Bioassay Technology Laboratory) according to the manufacturer's instructions.[(16)]

Total 90 recently delivered placentae of PE women (n=45) and normal women (n=45) were collected from the department of gynecology and Obstetrics immediately after delivery, transported to the examination site. All the placentae were washed and all extra placental membranes and umbilical cord of placentae were trimmed.[(17)]

Morphological examination:

The shape of the placenta was observed by appropriate assessment and every collected placenta was labeled oval or round whereas, the Laica (PS3001) Digital baby weighing scale was used to weight the placentae.[(18)] The volume of the placenta was measured by a measuring jug by water displacement method while the thickness was measured by incorporating a needle at the center of placentae. The placental diameter was measured by a plastic tape. The diameter measured first was considered as the first diameter later the second diameter was taken perpendicular to the first one. The mean of the two measured diameters was counted as the diameter of the placenta. Moreover, for the cotyledons, counting was started from the left to the right side of placenta and returning to the left side of placenta making a loop. Following this procedure, the entire number of cotyledons were counted and noted. Lastly, the feto-placental ratio calculated by division of the fetal weight to the placental weight.[(19)]

Microscopic Evaluation:

For microscopic examination, one section of each placenta was taken. The tissue samples were then well-kept-up in 10% formalin solution in a container labeled, capped and leak-proof for fixation for 24-48 hours. The specimens were trimmed to fit into tissue cassette after fixation. The filled tissue cassettes were stored in formalin till further processing. Later they were immersed in increasing concentrations of alcohol (50%, 75% and 90%) and up to (100%) alcohol for one hour in each step to remove the water and formalin from the tissue. Then the tissue was left in pure alcohol for further 12 to 15 hours. Specimen were infiltrated with paraffin wax for one hour at 60 degrees centigrade in oven so that the tissue was surrounded by a large block of molten paraffin wax for sections of 5µm thickness by using a rotary microtome.[(20)] Tissue sections were then stained with hematoxylin and eosin (H&E) and examined under light microscope (Nikon eclipse 50i microscopy) for evaluation of histological features.

The information was gathered and stored in SPSS ver. 22.0 was used to analyze the data. Comparison of placental features (continuous variables) between normal and pre-eclamptic mothers was done using independent sample t-test. While categorical variables were compared using Chi-square test. Significant level was set at $p< 0.05$.

RESULTS:

Total 90 patients were included in study with mean age of 27.22 ± 4.31 years. Most of the pregnant women had gravida between 2 to 5. Regarding the mode of delivery, most of the deliveries performed were normal 64.4% and 35.6% were performed through C- Section in control group. (Table I)

Table I: Demographic characteristics of study population (n=90)

		Control (n=45)	PE (n=45)	Mean± SD
Maternal age (in years)		27.64±3.718	26.87±5.012	27.26±4.405
Gravidity		2.98±1.406	3.84±1.609	3.41±1.564
Gestational age (in weeks)		38.92±.954	38.81±.875	38.87±.912
Socioeconomic status	Lower Middle Class	30(66.7)	41(91.1)	71(78.9)
	Middle Class	5(11.1)	-	5(5.6)
	Poor	10(22.2)	4(8.9)	14(15.6)
Mode of delivery	C-section	3(6.7)	31(68.9)	34(37.8)
	Normal	42(93.3)	14(31.1)	56(62.2)

The mean Betatrophin levels in women with normal pregnancy is presented in table II. There was a significant differences ($p<0.05$) in levels of BT in PE and Control female.

Table II: Comparison of Hormonal Assay among Normal and Pre-Eclamptic Cases

Variable	Control	PE	P-value
Betatrophin (µg/L)	1.66±0.10	1.71±0.06	0.008*

Gross morphological parameters were compared using independent sample t-test, there was a significant difference ($p<0.05$) in the mean weight, diameter, feto-Placental ratio, and number of cotyledons among control and pre-eclamsptic groups. (Table III)

Table III: Comparison of Gross Morphology of plancentae of Control and Pre-eclamptic (n=90)

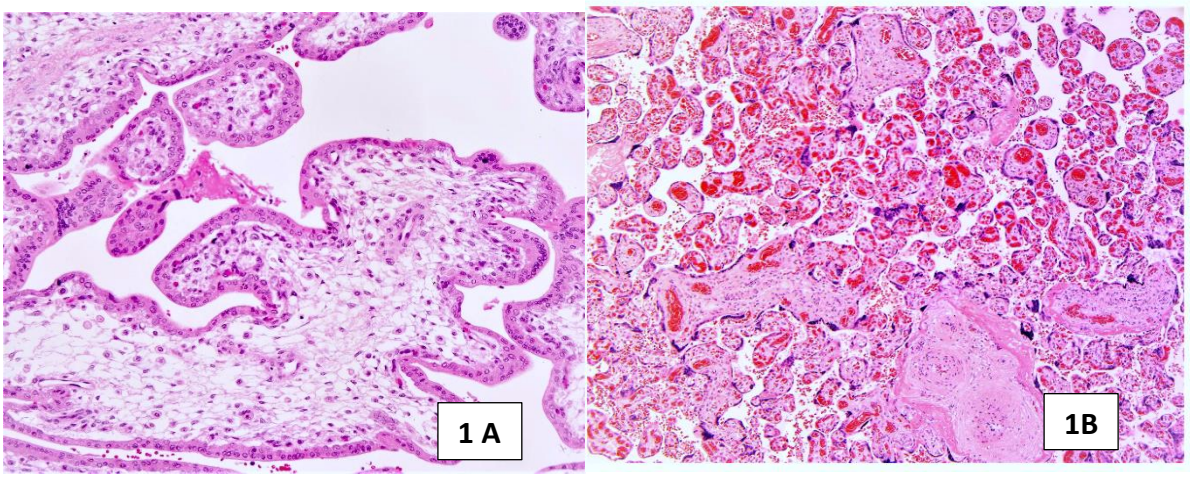
	Control	PE	p-value
	Mean± SD	Mean± SD	
Placental weight (g)	411.29±90.58	362.44±62.96	0.004*
Placental volume (ml)	450.33±59.74	413.33±53.72	0.003*
Placental thickness (cm)	2.53±0.43	2.16±0.54	0.000*
Placental diameter (cm)	19.26±1.70	16.79±4.46	0.001*
Feto-Placental ratio	6.82±0.19	6.52±1.23	0.102*
Number of cotyledons	17.13±1.93	15.44±2.65	0.001*

Table IV is demonstrating the microscopic features of placental tissues of PE and control patients. The frequency of syncytial knots, hypovascular villi, cytotrophoblastic proliferation and villous necrosis were significantly high among mothers with PE as compared to those with normal mother placenta.

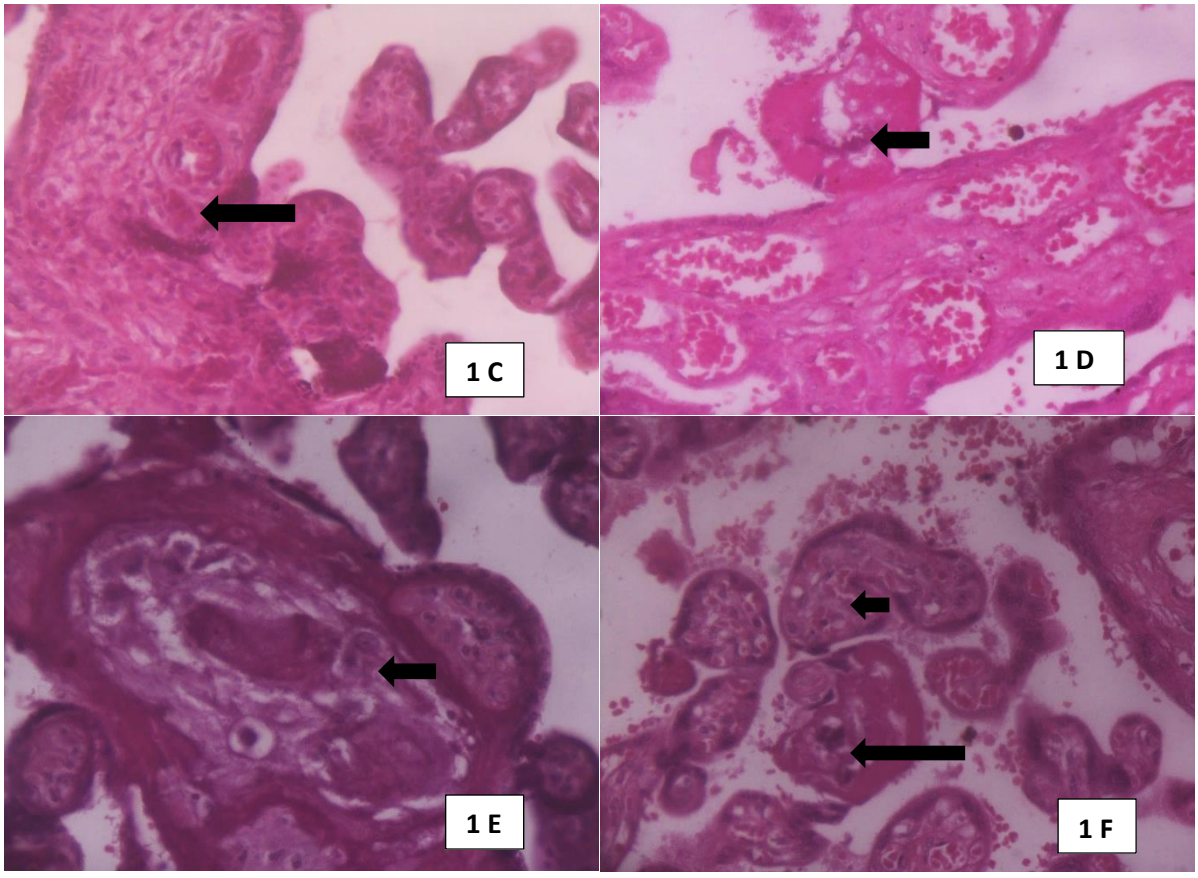
Table IV: Difference between Microscopic Features of Placenta among Normal and Pre-Eclamptic Cases

		Control	PE	p-value
		n(%)	n(%)	
Syncytial Knots	Not seen	32(71.1)	10(22.2)	0.001
	Present	13(28.9)	35(77.8)	
Hypo-vascular villi	Not seen	36(80.0)	16(35.6)	0.001
	Present	9(20.0)	29(64.4)	
Cyto-trophoblastic proliferation	Not seen	39(86.7)	13(28.9)	0.003
	Present	6(13.3)	32(71.1)	
Villous Necrosis	Not seen	30(66.7)	6(13.3)	0.001
	Present	15(33.3)	39(86.7)	

Photo micrographs of placental tissues of normal and PE mothers are depicted in figure 1 below. The photomicrographs 1A & B showing the normal placental tissue under light microscope 20X. The normal decidual plate is seen in 1A while stem villi, terminal and intermediate villi are seen in 1B.



Photomicrographs 1C-1F is presenting the placental tissue findings of PE mothers. In these, syncytial knots are seen in 1C (pointed by arrow) while in 1D the arrow is pointing the pervillous fibrin deposition. The syncytial knots and perivillous fibrin deposition is seen in 1E under 20X magnifications while arrow in 1F pointing syncytial knots and calcification.



DISCUSSION:

Preeclampsia is the life-threatening condition occurring in pregnancy. Initiation and aggravation of hypertension in pregnancy remains unknown. It is the leading cause of maternal and fetal death.[7] This cross-sectional study was designed to determine histological changes in the normal and PE women Placentae and its correlation with BT levels. This study examined 90 placentae of pre-eclamtic and normal mothers. Most of the pregnant women had gravidity between 2 to 5 and their mode of the delivery was mainly through C- Section in preeclamptic group while normal mode of delivery was seen in control group. Abbas et al also observed that the PE was more common in 24-28 years.[21] Another study by Obed SA et al concluded that PE is more common in nulliparous women

than 35 years of age.[22] Tyas et al and Cavazos et al. revealed that the PE is more common in advanced maternal age and cesarean delivery was also more often in PE.[23, 24]
Placental weight predicts the fetal birth weight because fetus depends on this organ via fetomaternal circulation; both are growing simultaneously. Current study revealed low mean placental weight in pre-eclamptic group than in control group. The placental weight was 483.29±14.94 in control group while placental weight in the preeclamptic group was 344.67±54.7. These findings are in line with studies conducted by Jansen et al. and Abbas et al. [8, 21]

Regarding the Microscopic changes pre-eclampsia group had significant changes in Syncytial knot, hypovascular villi, cytotrophoblastic proliferation and villous necrosis as compared to normal pregnancies. Na et al revealed that placentae from hypertensive mothers showed significant increase in syncytial knots, cyto-trophoblstic proliferation than non-hypertensive group.[12] Fisher et all showed increased syncytial knots, trophoblastic basement membrane thickening, and fibrin deposition is significant in preeclampsia.[25]

Betatrophin (ANGPTL8), predominantly produced by liver and adipose tissues. This hormone has the main role is in lipid metabolism. BT augments glucose tolerance during insulin resistance due to higher proliferation of pancreatic β-cells. This study investigated the correlation between betatrophin and placental histological changes in PE. PE groups were analyzed against the control group. Plasma betatrophin levels 1.66±0.10 in control group while in preeclampsia its levels 1.71±0.06 with the p-value of 0.008* were observed in this study. Significantly higher levels of BT was observed in preeclampsia. Similar findings are also reported by Akdamir et al. and Simsek et al. and reported that betatrophin can be an important biomarker for early management and diagnosis of PE.[26, 27]

This was cross-sectional and single centered study involved the limited number of placentae of pre-eclamptic and normal mothers. Serum markers specially anti-oxidative markers levels, serum glucose and insulin levels along with insulin resistant were not assessed which were our limitations. Lastly, effects of betatrophin on pregnancy outcome and other markers were not assessed.

CONCLUSION:

Placental morphological and morphometric parameters were more frequent in PE along with plasma elevated levels of betatrophin. These findings may bring out betatrophin as a new marker to predict, prevent, and treat pre-eclampsia.

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