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Histochemistry of Placental Lipids in Preeclampsia

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Abstract

Introduction: ***Physiologic pregnancy is associated with broad spectrum of metabolic adaptations which includes increased lipid metabolism and lipoproteins. Elevated lipid levels in first to third trimester may serve as energy store to fulfil maternal and fetal metabolic needs. Lipids are the major structural components of cell membrane thus are involved in cell replication. Changes in lipid metabolism in pregnancy ensure a continuous supply of nutrients to the growing fetus. Preeclampsia is a systemic disorder which affects 5 to 7 percent of women worldwide and is a major cause for maternal and neonatal morbidity and mortality. Altered placental lipid metabolism in preeclampsia could be a source of placental pathological changes in preeclampsia. The purpose of this study is to find out the exact histochemical localization of lipids in preeclamptic placentae and its correlation with growth and development of the fetus.***

Methods: ***50 normal and 50 preeclamptic placentae were collected immediately after delivery from Department of Obstetrics and Gynecology. Placentae were obtained from known preeclamptic consenting cases who had no history of hypertension before pregnancy or during first 20 weeks of gestation, who had consistently recorded systolic and diastolic blood pressure of 140 / 90 mm of Hg or above and proteinuria ≥ 300mg / 24 hrs. Placental lipid distribution was demonstrated by using Sudan black stain.***

Results: ***Fat distribution was more widespread in preeclamptic placentae as compared to normotensive placentae.***

Conclusion: ***Abnormal lipid metabolism may be involved in pathogenesis of preeclampsia. Altered lipid profile in preeclampsia can reduce membrane fluidity and disrupt transport across placental trophoblast. This could compromise the transport of nutrients to the fetus.***

Keywords: ***Preeclampsia, Pregnancy, Lipids, Placenta.***

Introduction

Placenta is a vital organ playing central role in pregnancy. It maintains pregnancy and promotes normal fetal development and serves as a major organ for transfer of essential elements between mother and fetus.[1] Preeclampsia is a systemic disorder defined as development of hypertension and proteinuria after 20 weeks of gestation in previously normotensive woman. Preeclampsia affects 5 to 7 percent of women worldwide and is a major cause for maternal and neonatal morbidity and mortality. Preeclampsia is often associated with intrauterine growth restriction. [2]

Physiologic pregnancy is associated with broad spectrum of metabolic adaptations which includes increased lipid metabolism and lipoproteins. Elevated lipid levels in first to third trimester may serve as energy store to fulfil maternal and fetal

metabolic needs. [3] Lipids are the major structural components of cell membrane. The role of placental lipid metabolism is therefore relevant to cell replication and immunological responses which may further be affected by changing pattern of blood lipids during gestation. Placental lipid metabolism may also be involved in mechanism conferring immune status on the conceptus. Changes in lipid metabolism in pregnancy ensure a continuous supply of nutrients to the growing fetus. Despite the importance of lipids in cell function their metabolism still remains doubtful in placental investigation. [4,5] Maternal hypertriglyceridemia in late gestation has an important role as a source of triglycerides for milk formation just before parturition.[5] During early pregnancy there is increased body fat accumulation and lipogenesis. In late pregnancy however, there is accelerated breakdown of fat depots which play a key role in fetal development. [6]

Pregnancy disorders such as preeclampsia are associated with dysregulation of lipid metabolism. Dyslipidaemia is a hallmark of preeclampsia which is often associated with increased maternal plasma levels of lipids. Various studies have focused on maternal lipid profile in preeclampsia, however there is scarce information on lipids in placental tissue and data for gestational diseases are lacking. Altered placental lipid metabolism in preeclampsia could be a source of placental pathological changes in preeclampsia. [3, 7] The purpose of this study is to find out the exact histochemical localization of lipids in preeclamptic placentae and its correlation with growth and development of the fetus.

Materials and Methods

Cross sectional study was conducted in Department of Anatomy of our medical college. Consecutive convenient sampling method was done. 50 normal and 50 preeclamptic placentae were collected immediately after delivery from Department of Obstetrics and Gynecology of our hospital. Institutional ethical committee clearance was taken. Written informed consent was obtained from all mothers participating in the study.

Samples were divided into two groups as Group A and Group B.

Group A:

Control group (Normotensive): Placentae were obtained from pregnant women who did not have any clinically detectable abnormalities. These women had normal blood pressure, no proteinuria and no oedema.

Group B:

Study group (Preeclampsia): Placentae were obtained from known preeclamptic cases who had no history of hypertension before pregnancy or during first 20 weeks of gestation, who had consistently recorded systolic and diastolic blood pressure of 140 / 90 mm of Hg or above and proteinuria ≥ 300 mg/24 hrs. Detailed menstrual and obstetric history and past history was obtained to exclude preexisting hypertension and other complications. Fetal weight, sex, any congenital anomaly and APGAR score at 1 and 5 minutes after delivery was recorded as parameters of fetal outcome.

Placental lipids were demonstrated by using Sudan black stain. Whole thickness tissue was cut out from central and peripheral part of placenta. Frozen sections of 10 micron thickness were taken from this fresh unfixed tissue. These were further stained by sudan black. 100 villi were studied from each of central and peripheral section of placentae for distribution of lipids.Fat distribution was observed in all the layers of villus irrespective of control or preeclamptic placentae. But the number of villi showing fat deposition was different in control and preeclamptic placentae. Hence quantification of lipids was assessed visually and was classified based on the extent of distribution of fat deposition in the number of villi to differentiate between control and preeclamptic placentae.

Results

Table 1: Maternal parameters of control and study group

	Blood Pressure (mmHg) Mean ± SD	
	Systolic	Diastolic
Control group	118±5.42	78.68± 3.56
Study group	156.36±14.98	98.84±6.06

P value < 0.05 – statistically significant

Table 2: Fetal parameters of control and study group

Fetal parameters	Control Group Mean ± SD	Study Group Mean ± SD
Birth weight (kg)	2.8±0.46	2.1± 0.39
APGAR score	8.9±0.76	6.5 ±0.95

P value < 0.05 – statistically significant

Under low power: Preeclamptic placentae showed widespread and increased deposition of fat per low per field as compared to control placentae. (Figure 1, 2)

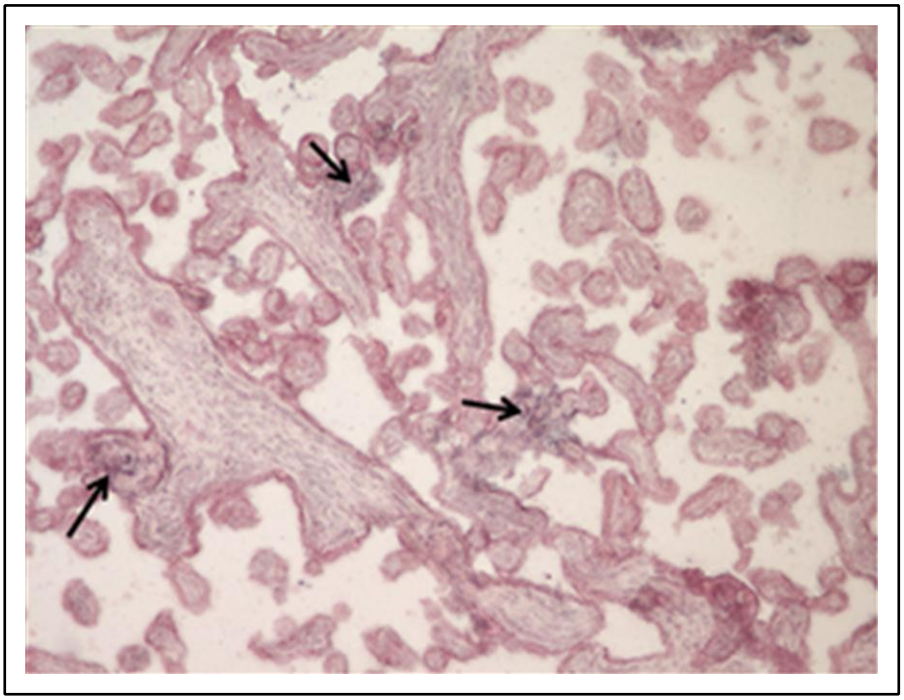

Figure 1 Control placenta. (10X) Arrows showing fat deposition

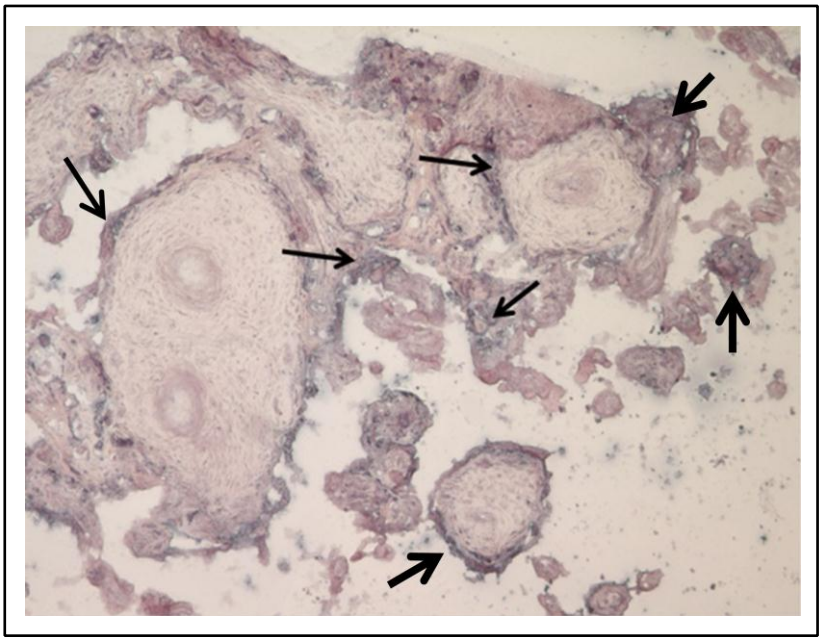

Figure 2 Preeclamptic placenta (10X)
Arrows showing increased and widespread fat deposits

Under high power: No particular pattern of fat deposition was observed.

In both control and preeclamptic placentae fat deposition was observed in :

1. Syncytiotrophoblast
2. Basement membrane
3. Connective tissue stroma

Fat deposition was assessed visually. Since no particular pattern of lipid deposition was observed classification was based on enzymatic activity observed in number villi showing fat deposition.

(+): Fat deposition in 1-25 villi
(++): Fat deposition in 26-50 villi
(+++): Fat deposition in 51-75 villi
(++++): Fat deposition in 76- 100 villi

Control Placentae: (Figure 1)

(+): Fat deposition was observed in central section of **30(60%)** and peripheral section of **29(58%)** placentae.

(++): Fat deposition was observed in central section of **17(34%)** and peripheral section of **20(40%)** placentae.

(+++): Fat deposition was observed in central section of **3(6%)** and peripheral section of **1(2%)** placentae.

Preeclamptic Placentae: (Figure 2, 3)

Preeclamptic placentae showed high intensity widespread fat deposition as compared to control placentae. **(**Figure 3)

(+++): Fat at deposition was observed in central section of **12(24%)** and peripheral section of **10(20%)** placentae.

(++++): Widespread and increased fat deposition was observed in central section of **34(68%)** and peripheral section of **37(74%)** placentae.

(++) However, central section of **4(8%)** placentae and peripheral section of **3(6%)** placentae showed lipid distribution similar to control placentae.

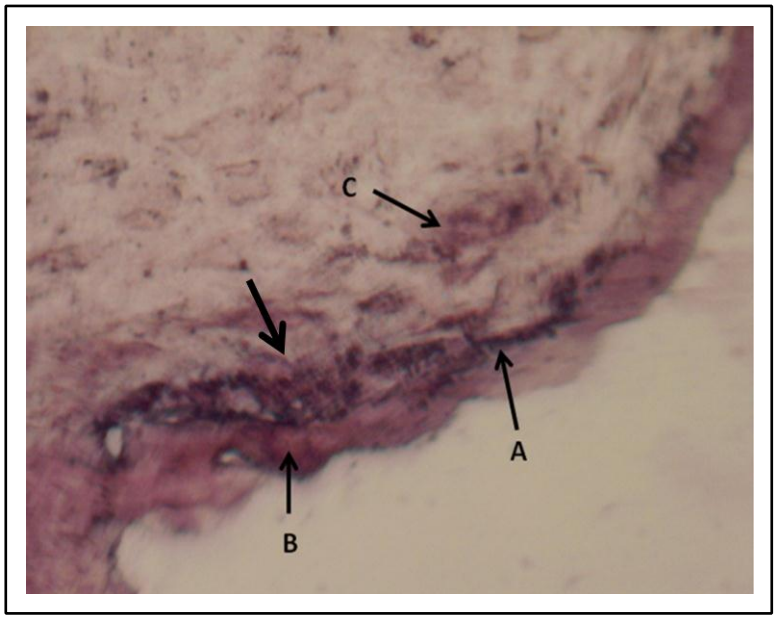

Figure 3 Preeclamptic placenta (100X)
Arrows showing increased intensity of fat deposition
A-Basement membrane, B-Syncytium, C-Connective tissue stroma

Table 3: Distribution of lipids

Lipid distribution	Control placentae		Preeclamptic placentae	
	Central section	Peripheral section	Central section	Peripheral section
+	30 (60%)	29(58%)	-	-
++	17(34%)	20(40%)	4 (8%)	3(6%)
+++	3 (6%)	1(2%)	12 (24%)	10 (20%)
++++	-	-	34(68%)	37(74%)

Disscussion

Placental transport of lipids to the fetus involves metabolic alterations in the placenta and release into fetal plasma.[8] During early gestation maternal cholesterol is important source of cholesterol for the fetus , however its importance becomes minimal during late pregnancy due to high capacity of fetal tissues to synthesise cholesterol.[6] The concentration of phospholipids is about 75% of total placental lipids. It has been suggested that placental phospholipids are involved in transport of amino acids across placenta. [4]

Previous workers mainly focused on maternal lipid profile in pregnancy. Kalar et al[5] found high lipid levels in maternal circulation of preeclamptic cases as compared to normotensive controls. Lima et al[9] reported higher levels of triglycerides in maternal serum of preeclamptic patients as compared to healthy controls. Robinson et al[10] reported that elevation of free fatty acids in maternal circulation in preeclampsia may be involved in pathogenesis of preeclampsia.

However, negligible data is available on lipids in placental tissue. So far limited numbers of studies have investigated the fat content of placental tissue in preeclampsia. Huang et al[3] are the only one to study distribution of lipids in placental tissue of control and preeclamptic placentae. In present study fat accumulation was identified histochemically using sudan black stain. Increased and widespread reactivity of sudan black was observed in preeclamptic placentae as compared to control placentae. Increased fat deposition was observed on syncytial basement membrane, cytoplasm and villous connective tissue stroma. Similar results have been reported by Huang et al.[3] They reported 30% increase in fat deposition in preeclamptic placental tissue as compared to control placentae.

Preeclampsia is a pregnancy specific disorder that adversely affects maternal vascular function and fetal intrauterine growth. It is a major cause of maternal and fetal morbidity and mortality.[2] In preeclampsia placental damage appears to trigger the maternal syndrome. Hypertension and organ damage are mediated by endothelial dysfunction. Endothelium seems to be the target of factors produced by damaged placenta. Thus abnormal placentation in preeclampsia induces endothelial dysfunction and is responsible for maternal syndrome.[11]Association of altered serum lipid profile and preeclampsia has been reported earlier .Functions of various organs involved in lipid and lipoprotein metabolism are known to be affected by preeclampsia and related disorders. Several studies have shown that endothelial dysfunction is related to hyperlipidemia.[5,12] Present study showed abnormal fat deposition in preeclamptic placentae. Thus abnormal lipid metabolism seems to be a source of endothelial dysfunction and pathogenesis of preeclampsia.

Hererra et al[13] reported that enhanced oxidative stress in pregnancy may be related to maternal hyperlipidaemia . Previous workers like Siddiqui et al[14], Maarten et al[15] have reported enhanced oxidative stress and lipid peroxidation in preeclampsia. Increased oxidative stress produces lipid peroxides which are highly reactive compounds that can have direct interactions with cell membrane and cause cellular dysfunctions.[16] It has been demonstrated that placental production of lipid peroxides is abnormally increased in preeclampsia which cause endothelial injury and dysfunction.[17] Staff et al[18] reported increased total cholesterol, phospholipids and lipid peroxides in decidua basalis of preeclamptic samples as compared to samples from normotensive controls. They suggested that maternal endothelial dysfunction in preeclampsia could be due to elevated lipid content in decidua basalis tissue.[18] Preeclamptic women present arterial lesions at uteroplacental implantation sites. Changes in lipid metabolism in preeclampsia may contribute towards endothelial lesions.[9,5] Increased phospholipids in preeclamptic placentae could be a source of lipid compounds that cause oxidative damage within placental tissue and may be associated with pathophysiology of placenta in preeclampsia.[3]

Transplacental nutrient transport from mother to fetus is mediated across basal plasma membrane of syncytiotrophoblast.[19] Placental activities such as transport , permeability, activities of enzyme and stability are greatly influenced by the physical state of membrane lipid bilayer and protein lipid interactions. Cholesterol helps to maintain the fluidity of cell membranes. A change in cholesterol to phospholipid ratio in placenta affects placental function and transport. Cholesterol can effectively modulate the physical state of phospholipid bilayer and can lower membrane fluidity.[20] Previous workers have reported that under normal physiological conditions cholesterol phospholipid ratio of syncytial membranes decreases during pregnancy, thus membrane fluidity increases.[20,21] In preeclampsia however it has been reported that

syncytiotrophoblast has decreased fluidity.[22] Huang et al reported increased mean cholesterol in preeclamptic placentae as compared to normal control placentae. Solomon et al [23] have reported that high cholesterol levels are associated with preeclampsia. Thus these increased levels in preeclampsia can reduce membrane fluidity by altering cholesterol phospholipid ratio and therefore disrupt transport across placental trophoblast[.3] In present study the mean fetal birth weight for control group was 2.8 kgs and study group was 2.1 kgs. There is evidence that suggests that in preeclampsia major involvement of maternal lipid metabolism in fetal growth exists, however exact mechanism is unknown.[24] Impaired lipid metabolism and placental transport seem to contribute to decreased fetal birth weight.

In present study increased reactivity of sudan black was observed in preeclamptic placentae as compared to control placentae. This demonstrates increase in phospholipids in preeclamptic placental tissue. Presence of hypercoagulation in the placental circulation has been one of the hypothesis associated with pathogenesis of preeclampsia.[3] It has been suggested that phospholipids may be involved in the intravascular coagulation associated with toxaemia of pregnancy.[4,25,26] Thus increased phospholipids may activate blood coagulation and may contribute to the development of preeclampsia.

Conclusion:

Present study demonstrated higher fat content in preeclamptic placentae as compared to control placentae. Thus it is suggested that abnormal lipid metabolism may be involved in pathogenesis of preeclampsia. Altered lipid profile in preeclampsia can reduce membrane fluidity and disrupt transport across placental trophoblast. This could compromise the transport of nutrients to the fetus and may be one of the causes of low fetal birth weight. However this needs further investigation.

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