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Research Paper

Placental Apoptosis and its Correlation With Severity of Disease in Preeclampsia and Eclampsia

Medical Science

KEYWORDS : Preeclampsia, Eclampsia, Placental Apoptosis, Biochemical markers.

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ABSTRACT

To determine placental apoptosis in pregnancies complicated with preeclampsia and eclampsia and to correlate it with the severity of disease process. A Prospective cohort study in pregnant women attending the antenatal clinic and emergency services in University teaching hospital, Institute of Medical Sciences, Varanasi, India.

The placental apoptosis was determined by calculating the apoptosis (%) through TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) assay.Placental apoptosis was significantly higher in pre-eclampsia and eclampsia as compared to controls ($p<0.001$). Placental apoptosis and systolic blood pressure ($R2 = 0.669$) and diastolic blood pressure ($R2 = 0.796$) had a linear correlation. The level of biochemical markers correlated with each other but not with the extent of apoptosis in cases.Increase in apoptosis correlated with rise in systolic and diastolic blood pressure and severity of disease process.

The placenta is a temporary organ that undergoes growth and development followed by senescence and death in nine months. The process of apoptosis (programmed cell death) was first described in 1972 by Kerr et al. Apoptosis is an active, regulatory response of inducible cells to specific stimuli that occurs only in cells having the relevant response pathways[1]

Recently, a number of studies have suggested that apoptosis plays a role in the normal development, remodeling, and aging of the placenta.[2] Villous cytotrophoblasts proliferate, differentiate, and merge into the syncyticial layer by fusion. Aged syncytioplasm and nuclei are focally isolated within syncytial buds and are shed into the maternal circulation as synctial knots/ sprouts. Most of these aged nuclei are in some stage of apoptosis. Trophoblast turnover in villous tissue changes throughout normal pregnancy,[3] and some observation suggest that the rate of trophoblast apoptosis may change under certain pathologic conditions, such as preeclampsia or intrauterine growth retardation.[4]

There was a significant increase in the incidence of apoptosis in placental samples obtained from second and third trimesters as compared with those obtained from the first trimester.[3]

The role of placenta in the pathophysiology of pre-eclampsia is strongly supported by the rapid resolution of symptoms after delivery of placenta. Apoptosis, as programmed cell death, is essential for homeostasis of human tissues including the human placenta. Placental apoptosis is the key intermediary event in the generation of the syndrome of Pre-eclampsia and Eclampsia.[5]

Preeclampsia is associated with abnormal cytotrophoblast differentiation, shallow invasion, and decreased blood flow to the placental. To determine whether abnormal differentiation and /or hypoxia leads to cytotrophoblast apoptosis, we used the TUNEL (terminal deoxynucleotidyl transferase mediated dUTP nick end labeling) method to label DNA strand breaks in tissue sections the placenta.[6]

The study is an attempt to determine the degree of placental apoptosis in pregnancies complicated with pre-eclampsia and eclampsia and to correlate it with the severity of disease process.

Material and Methods

This prospective cohort study was conducted on 49 pregnant women admitted in Department of Obstetrics and Gynecology, Institute of Medical Sciences, Varanasi, India.

Selection criteria: Patients attending the antenatal clinic were evaluated and screened and those who fulfilled the criteria for Pre-eclampsia, as defined by National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy (2000)[7], were taken as cases, study also includes seven cases of eclampsia admitted through emergency services. The control group comprised of normotensive (BP<140/90 mmHg) antenatal patients. Patients having medical complications like chronic hypertension, renal disease and diabetes were excluded.

Sample Collection: Placental biopsy specimens were collected by direct visualization of the maternal surface of placenta following delivery and transported immediately to the laboratory in Saline-Citrate solution. Cells were isolated from Placenta and the apoptosis was analyzed by APO-DIRECT kit (BD Biosciences Pharmingen Catalog number 556381). One of the later steps in apoptosis is DNA fragmentation, a process which results from the activation of endonucleases during the apoptotic program. The APO-DIRECT™ assay is a single-step method for labeling DNA breaks with FITC-dUTP (fluorescein isothionate) followed by flowcytometric analysis according to the technique described by Douglas et al (1998)[8]. Graphs.1 and 2 depicts how apoptosis was calculated in control and cases (eclampsia) respectively. Gate M1 exhibited normal cells whereas Gate M2 recorded apoptotic cells.

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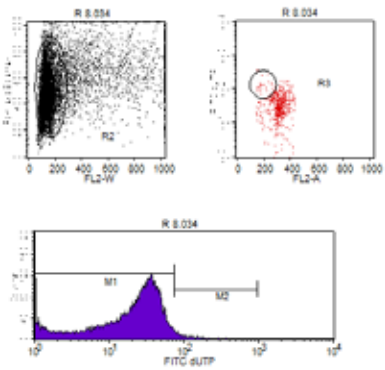
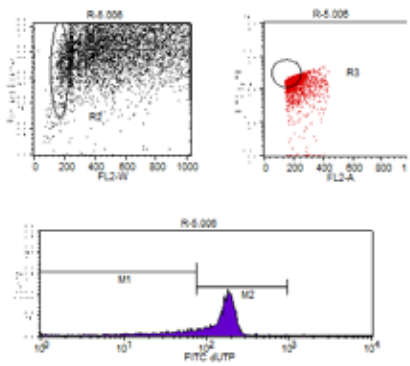
Graph. 1



Graph. 2



Biochemical Tests: These were performed in all cases and control. The tests include-Serum creatinine, uric acid, Liver Function Tests, LDH and Platelet count.

Statistical Analysis: SPSS 16 Software was used for statistical analysis

Results

The present study was carried out on 49 antenatal women, out of which 21 normotensive women were taken as controls and 28 pregnant patients with Pre-eclampsia (21) and eclampsia (7) as cases.

Women in both groups were similar with regard to age, parity, geographical area and socioeconomic status (Table 1)

Table 1. Demographic Data

		Control (n = 21)	Cases (n = 28)	P
Age (yrs)		24 .38 (±3.32)	25.52 (±4.52)	NS*
Parity	Nullip-arous	10	15	NS†
	Multip-arous	11	13	NS†
Mean systolic blood pressure (mmHg)		124(±14.03)	157 (±20.99)	<0.001*
Mean diastolic blood pressure (mmHg)		77 (±11.57)	106 (±12.71)	<0.001*
Vaginal delivery	Spontaneous vaginal delivery	13	6	<0.01†
	Induction	-	6	<0.05†
LSCS		8	16	NS†
Mean gestational age (days)		270 (±11.37)	254 (±20.68)	<0.01*
Mean baby birth weight (gms)		2702 (±412.28)	2043(±627.94)	<0.001*

*student t-test ; † Z- test

The mean systolic and diastolic blood pressures of cases were significantly higher as compared with control. Gestational age and mean birth weights of babies were significantly lower in cases than in control patients.

Percentage apoptosis in preeclampsia (64.32 ± SE 4.19) and eclampsia (88.69 ± SE 3.05) were significantly higher (p<0.001) than control (18.35 ± SE 3.14) (Table 2).

Table 2.Apoptosis in cases (Preeclampsia and Eclampsia) and controls

	Mean Apoptosis %	Standard error of mean
Eclampsia	88.69*	3.05
Pre-eclampsia	64.32*	4.19
Control	18.35	3.14

*p <0.001

On linear regression analysis significant correlation was seen between Apoptosis (%) and Systolic and Diastolic Blood Pressure with r^2 values are 0.669 and 0.796 respectively.

Apoptosis was significantly higher in patients with systolic blood pressure ≥180 mm Hg. and diastolic blood pressure ≥110 mmHg. Placental apoptosis did not correlate with the level of biochemical markers (Table 3).

Table 3. Linear CorrelationCoefficient of Apoptosis With study variables

Variable	Control	Cases
Systolic BP	0.588*	0.744*
Diastolic BP	0.680*	0.788*
Gestational age	0.022	0.067
Baby weight	0.240	0.233
Uric acid	0.327	0.427
Serum creatinine	0.306	0.264
Lactate Dehydrogenase	0.326	0.194
Platelet count	0.235	0.437*
ALT	0.189	0.332
AST	0.207	0.080

*p<0.001

Discussion

Apoptosis is mediated by diverse mechanism commonly divided into the ligand receptor pathway and the mitochondrial pathway. Tumor necrosis factor α was the first cytokines identified to induce the apoptosis through the ligand receptor pathway in the cultured human trophoblast.[9] Hypoxia enhances the apoptosis in cultures of human trophoblasts and that the enhanced apoptosis is associated with increased expression of the proapoptotic protein p53 and Bax and with lower expression of the antiapoptotic protein Bcl-2.[10]

Trophoblast apoptosis occurs in normal placenta throughout pregnancy but with higher frequency near term in comparison to first trimester.[3] Placental apoptosis acts as a trigger for initiating maternal systemic inflammatory responses. We compared the placental apoptosis in normal pregnancy and preeclampsia patients. We found significantly increased placental apoptosis in

pre-eclampsia (p<0.001) and eclampsia (p<0.001) compared with control (Table 2). The disease was fulminant in majority of the cases of Preeclampsia with mean apoptosis (%) 70.42 ± SE 3.79. The apoptosis indicates extensive placental damage and results in intrauterine growth restriction.

The increased placental apoptosis in Preeclampsia may lead to the deportation of placental debris into the maternal circulation which results in maternal systemic inflammatory response and clinical signs and symptoms of the Preeclampsia (Sibai and Dekker, 2005)[5]. It appears logical to conclude that higher the placental apoptosis higher will be the maternal response and severity of the disease. The apoptosis exhibited a close linear correlation with systolic blood pressures and diastolic blood pressure.

Oxidative stress may increase the placental apoptosis (Redman and Sagent 2003)[11].Hypoxia/Reoxygenation induced Apoptosis in the Syntiotrophoblast was studied by Belkacemi et al (2007)[12] and it was observed that the increased number of TUNEL- positive nuclei was paralleled by higher levels of 4-hydroxynonenal (marker of lipid peroxidation). Treatment with glyceril trinitrate during the hypoxia/ reoxygenation cycle blocked the increase in the number of TUNEL-positive nuclei.

Some antioxidants not only detoxify free radicals but also inhibit apoptosis, and may have anti-inflammatory properties (Takacs et al., 2003)[13].There is an emerging consensus that antioxidant therapy in cases of preeclampsia can slow the rapid progression of disease to severe preeclampsia and eclampsia and result in the decrease in the related morbidity and mortality. The present study suggests a reasonable basis for the trial of antioxidants in cases of Preeclampsia to prevent rapid progression of disease process, decrease the severity of disease and related hypertensive complications. However, this was not substantiated by other studies.

In recent studies by polymerase chain reaction, cell free fetal DNA can be detected in maternal plasma (Lo and colleagues, 1997).[14] Holzgreve and associates (1998)[15] reported that fetal maternal cell trafficking is increased in pregnancies complicated by preeclampsia. It is hypothesized that free DNA is released by accelerated apoptosis of cytotrophoblasts (Di Federico and colleagues, 1999)[6] .It is hoped that in future cell free fetal DNA in maternal blood can become an important indicator of fetal well being in cases of preeclampsia.

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