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Role of Placental Mitochondria in Development of Pre-eclampsia: Focus on Mitochondrial Dynamics, Redox Signaling and Apoptosis

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Keywords

- Pre-eclampsia
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- Dynamin-related protein 1 (DRP1)
- Nitrosative stress

Abstract

Background: Mitochondrial dysfunction has been incriminated in the pathogenesis of pre-eclampsia (PE). This study aimed at evaluating the contribution of mitochondrial dynamics, biogenesis, redox signaling and apoptosis in the pathogenesis of mitochondrial dysfunction in pre-eclamptic placenta. Forty pregnant females were classified equally into two groups: Group I (control group), included normotensive pregnant females and Group II (PE group) included, pre-eclamptic pregnant females. After delivery, placental tissue samples were collected for estimation of mRNA expression levels of Mitofusin2 (Mfn2) using quantitative real-time PCR . Dynamin related protein 1 (Drp1), mitochondrial Cytochrome c release ,and 3-nitrotyrosine (3-NT) were measured by ELISA . Mitochondrial complex I, and citrate synthase enzyme activity were assessed spectrophotometrically. ATP levels, Caspase-9 activity , inorganic nitrites and nitrate levels ,and superoxide dismutase (SOD) activity were measured by colorimetric assay kit. Pre-eclamptic placentae showed significant decrease in complex I, ATP levels and citrate synthase activity. mRNA expression of Mfn2 were downregulated with marked elevation of DRP1 protein levels .There were altered redox status as judged by the elevation of NO and protein nitration with reduction in the total SOD activity in pre-eclamptic placentae. There were also activation of the mitochondrial pathway of apoptosis as judged by release of cytochrome c from the mitochondrial intermembrane space into the cytosol and significant increase in caspase-9 levels in Pre-eclamptic placentae compared to controls. Our data strongly nominated significant association between mitochondrial dysfunction, disturbed dynamics, altered redox status and the susceptibility to apoptosis in pre-eclamptic placenta as key players in the multifactorial pathogenic mechanisms of PE

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Introduction

Pre-eclampsia (PE) is a serious complication that occurs during the second and third trimester of pregnancy and affects the health of both mother and fetus. It is a rapidly progressive condition characterized by proteinuria, hypertension and edema[1]. Despite advances in the management of preeclampsia, it is still the main cause of maternal and perinatal mortality, complicating 5–10% of pregnancies worldwide [2]. Although intensive research has been performed on the molecular changes associated with PE, the etiology and pathogenic mechanisms of PE remain unclear. [3].

PE is thought to be associated with impaired trophoblast invasion into the myometrial segment of the spiral artery. Subsequent disturbance of placental oxygenation results in permanent ischemia/reperfusion and induction of oxidative stress in the placenta and maternal blood [4].

Even in normal pregnancies, placental mitochondrial dysfunction was found to increase significantly as pregnancy progresses, suggesting that it may play a role in the normal development and aging of the placenta[5]. Studies that have focused on the mitochondrial apparatus and antioxidant system in PE are very few, despite them being highly interconnected [6].

Mitochondria are dynamic organelles that constantly fuse and divide. These processes of fission and fusion (collectively termed mitochondrial dynamics) are important for mitochondrial inheritance and for the maintenance of mitochondrial functions. Mitochondria are responsible for producing most of the cellular ATP through oxidative phosphorylation[7] . Cell survival depends on mitochondrial fusion and division, possibly due to the high ATP demand required for specialized cellular morphological transformations. The imbalance in mitochondrial dynamics compromises the energy production and is linked to several diseases [8]. Research on mitochondrial dynamics gained much attention, as it is important for our understanding of many biological processes, including the maintenance of mitochondrial functions, apoptosis and ageing [9].

Mitofusin 2 (Mfn2) is a large GTPase required to tether the mitochondria together to initiate the process of fusion .It is present in the outer mitochondrial membrane and is involved in the maintenance of the mitochondrial network and bioenergetics. Mfn2 has a potential role in regulating cell proliferation and oxidative metabolism in many cell types. Mfn2 has been reported to be an important biomarker and therapeutic target molecule for cardiovascular diseases such as hypertension[10], however its role in the pathophysiology of PE remain unclear.

Mitochondrial fission causes organelle fragmentation and is increased in apoptosis, cell division, and is required for mitophagy [11]. Central to mammalian mitochondrial fission is the dynamin-related protein 1 (DRP1) which is a cytoplasmic large GTPase that mediates the fragmentation of mitochondria and peroxisomes. Given its cytoplasmic localization, several mechanisms exist to facilitate Drp1 translocation to mitochondria, especially upon mitochondrial dysfunction that is a key inducer of fragmentation[12].

Mitochondria are considered central integrators and transducers for pro-apoptotic

signals, forming the nexus between the non-specific inducer phase and the final execution phase of apoptosis[13]. Numerous cell stressors; such as hypoxia, ischemia, oxidative stress; are capable of inducing the mitochondrial pathway of apoptosis in PE. They induce permeabilization of the outer mitochondrial membrane with subsequent mitochondrial dysfunction and collapse of the inner membrane gradient, ending with the morphological and biochemical changes associated with apoptosis[14] .

Oxidative stress and mitochondrial dysfunction underlie the development of many pathological conditions as PE. Mitochondria are the major source of ROS because of their key role in ATP generation. NAD(P)H oxidases are a major source of superoxide in neutrophils and vascular endothelial cells and have also been reported in human trophoblast [15]. Peroxynitrite is a potent pro-oxidant which results from superoxide and nitric oxide interaction and affects many physiological functions. The peroxynitrite anion is extremely unstable thus evidence of its formation in vivo has been indirect via the occurrence of nitrated moieties including nitrated lipids and nitrotyrosine residues in proteins. Formation of 3-nitrotyrosine (protein nitration) is a “molecular fingerprint” of peroxynitrite formation[16].

The present study was designed to investigate the role of the antagonistic and balanced activities of the fusion and fission machineries, apoptosis, and nitrosative stress in mitochondrial dysfunction in placenta of patients with PE, in order to clarify some of the conflicting data in the literature on Mfn2 and DRP1 in PE, and try to find a relation between mitochondrial fission/fusion balance and the disease pathogenesis.

Subjects and methods:

Subjects:

The study population consisted of forty women who were receiving prenatal care at Obstetrics and Gynecology Department, Tanta University Hospitals during the period from Mars 2016 till December 2016. The subjects were divided into 2 groups: **Group I (control group):**included 20 normotensive pregnant women , were randomly selected and gestationally age matched.They were delivered prematurely (weeks 27-36) by caesarean section due to various complications other than PE including premature rupture of membranes, placental abruption or placental praevia . **Group II (PE group):** included 20 pregnant women with preeclampsia which was diagnosed according to the modified American College of Obstetricians and Gynecologists criteria [1] as follows: a blood pressure140/90 mm Hg on 2 occasions 2 hours to 2 weeks apart and proteinuria of 300 mg/24 h or, qualitatively, > 1+ by urine dipstick measurement after 20 weeks of gestation. All pre-eclamptic patients were delivered prematurely (weeks 27-36) by caesarean section. Exclusion criteria were preexisting chronic hypertension, diabetes mellitus, collagen vascular disease, renal disease, multiple gestations, and the presence of fetal malformations. The study protocol was approved by the Local Research Ethics Committee, Tanta University (Approval code 2806/2/16) and was in accordance with the principles of the Declaration of Helsinki II. A written informed consent was taken from all subjects included in this study. After enrollment of subjects, routine laboratory studies were performed

preceding labor and included urinary protein estimation, complete blood cell count, coagulation profile, liver function tests, serum creatinine, blood urea nitrogen (BUN), and uric acid level. After delivery, the placental weight was recorded, and placental tissue samples were collected.

Methods:

Placental tissue sampling: All placental tissues were obtained immediately after delivery. The samples were first thoroughly washed with cold phosphate buffered saline (PBS), and then the villous tissues beneath the chorionic and basal plates were quickly dissected, sliced into small pieces, snap-frozen in liquid nitrogen, and stored in−80°C freezer.

Placental tissue homogenization and Isolation of mitochondria and cytosol: Mitochondria and cytosol were isolated by differential centrifugation as previously described [17]. 0.5 g of of placental specimen was placed in a medium containing 0.25 M sucrose, 10 mM Tris-HCl, and 1 mM of the chelator EGTA, pH 8 at 4°C. The tissue was scissor minced and homogenized on ice using a Teflon Potter homogenizer. Placental tissue homogenate was centrifuged at 1,000 × g for 5 min to pellet cell fragments, and the supernatant was centrifuged at 9,500 × g for 10 min to pellet the nuclei. The supernatant was further centrifuged at 14,000 × g for 25 min to obtain the mitochondrial fraction and the resulting supernatant was used as the soluble cytosolic fraction.

Biochemical analysis:

Total proteins assay: Concentrations of total proteins in the samples were determined according to the method of Bradford with bovine serum albumin as a standard (#Cat no.500–0006, Bio\Rad Protein Assay)[18].

Assessment of mitochondrial function:

Spectrophotometric assay of mitochondrial complex I(NADH-Ubiquinone (CoQ) oxidoreductase enzyme, EC 1.6.5.3) activity: It was measured according to method of Birch-Machin et al.[19] by following the decrease in the absorbance due to oxidation of NADH at 340 nm, using extinction coefficient 6.811/mmol/cm.

Direct measurements of mitochondrial ATP levels: ATP levels were quantified using a colorimetric ATP assay kit (#Cat. No.K354-100, BioVision, Mountain View, CA, USA) according to the manufacturer's instructions.

Activity of the mitochondrial citrate synthase enzyme (EC 4.1.3.7): Citrate synthase activity was measured in a subsample of the isolated mitochondria as previously described[20]. Briefly, after thawing, a reaction medium containing 0.1 mM dithionitro benzoic acid (DTNB), 0.5 mM oxaloacetate, 50 µM EDTA, 0.31 mM acetyl coenzyme A, 5 mM triethanolamine hydrochloride and 0.1 M Tris–HCl was mixed and preheated for 5 min at 30 ◦C. Subsequently 10 µl of mitochondria were added to the reaction medium and citrate synthase activity was assessed spectrophotometrically at 412 nm and expressed as micromole per minute per milligram of protein.

Quantitative Determination of dynamin related protein (Drp1) levels: Drp1 levels were determined using sandwich ELISA kit (#Cat. No.MBS1272929, MyBioSource, CA, USA) according to the manufacturer's instructions. Concentrations were calculated using a standard

curve generated with specific standards provided by the manufacturer.

Quantitative measurement of Mitofusin2 (Mfn2) mRNA in Placental Villous Tissues by quantitative real-time reverse transcription PCR (RT-PCR):

RNA extraction: Total RNA was extracted from Placental Villous Tissues tissue using Qiagen RNeasy Mini Kit according to the manufacturer protocol. Total RNA concentration and purity were determined by measuring OD260 and OD260/280 ratio, respectively, on a NanoDrop spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA), RNA was then stored at -80°C.

cDNA synthesis: Total RNA samples were reverse-transcribed using the RevertAid H Minus First Strand cDNA Synthesis kit (Cat#K1632, Thermo Scientific Fermentas, St. Leon-Ro, Germany) according to the manufacturer's instructions. Briefly, ten µl of random hexamer primers (Roche, Mannheim, Germany) were added to 21 µl of RNA which was denatured for 5 minutes in the thermal cycler (Biometra, USA). The RNA-primer mixture was cooled to 4°C. The cDNA master mix was prepared (5 µl of first strand buffer, 10 mM of dNTPs,1 µl of RNase inhibitor, 1 µl of reverse transcriptase Superscript™ II-RT enzyme and 10 µl of DEPC treated water) according to the kit protocol and was added to each sample. The total volume of the cDNA master mix was 19 µl for each sample. This was added to 31 µl RNA-primer mixture resulting in a reaction volume of 50 µl, which was then incubated in the programmed thermal cycler one hour at 42°C, followed by inactivation of enzymes at 95 °C for 5 min, and finally cooled at 4°C for 5min The RNA was reverse transcribed into cDNA which was then stored at -20°C until used for PCR.

Real-time quantitative PCR: One µl of the cDNA was added to 20 µl reaction mixture of the QuantiTect SYBR-Green PCR kit (Qiagen) and 0.5 µM from the specific primer pairs for Mitofusin2 (Mfn2) (GenBank accession No. NC_000001.11). This cDNA was then amplified using the Step One instrument (Applied Biosystems, USA) as follows: Initial denaturation at 95˚C for 5 minutes was followed by 30 cycles with denaturation at 95˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 30 sec. A control reaction without a DNA template was performed in parallel to detect genomic DNA contamination.

The primer sequences for Mfn2 were designed according to **Dasgupta et al.,** [21] as follows: (forward) 5'-CTGCTAAGGAGGTGCTCAA-3′, and (reverse)5′-TCCTCACTTGAAAGCCTTCTGC-3′. The sequences of primers for 18S rRNA internal control were 5′-GTAACCCGTTGAACCCCATT-3′ (forward) and 5′-CCATCCAATCGGTAGTAGCG-3′ (reverse).The determination of the relative levels of gene expression was performed using the comparative cycle threshold (ΔΔCt) method and normalized to the reference gene 18S rRNA, which was not altered by the experimental conditions.

Assessment of apoptotic markers:

Mitochondrial Cytochrome c release:

Cytochrome c concentrations in both the mitochondrial and the cytosolic fractions were determined using the quantikine human cytochrome c ELISA kit (R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's

recommendations. The cytochrome c values were expressed as ng/mg protein.

Caspase-9 activity: The activity of caspase-9 was assayed in the supernatants of placental tissue homogenate using colorimetric assay kit (#Cat. No.BF10100, R&D) according to the manufacturers' protocol. Caspase-9 substrate is composed of the chromophore ρ-nitroaniline (ρNA) conjugated to a synthetic tetrapeptide LEHD (Leu-Glu- His- Asp). Cleavage of the peptide by the caspase releases free ρNA, resulting in a yellow color that was quantified spectrophotometrically at 405 nm using a microplate reader (Stat Fax 2100, NY, USA). Values were expressed as fold increase in caspase activities compared with that of controls.

Oxidative (Nitrosative) stress parameters:

Measurement of placental inorganic nitrites and nitrate: Nitric oxide production was determined by the evaluation of its oxidation products nitrites and nitrates using a Colorimetric nitrate/nitrite assay kit (#CAT: K262-200, BioVision, CA, USA) according to manufacturers' protocol. The absorbance was read at 540 nm .

3-nitrotyrosine (3-NT) levels: 3-nitrotyrosine is a surrogate index of peroxynitrite mediated protein oxidation/nitration. 3-NT levels of were determined in placental tissue homogenates using ELISA kit (#CAT:NWK-NTR01-02, Northwest, Life Science Specialties, Vancouver, Canada) for human nitrotyrosine, following the manufacturer's instructions. The final result was normalized to protein concentration and expressed as nmol/mg of protein.

Superoxide dismutase (SOD) activity: Total SOD activity was assessed using a colorimetric Assay Kit (BioVision, CA, USA) according to the manufacturer's protocol. This method is based on the use of a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Results were expressed as units of SOD per milligram of protein (U/mg protein).

Statistical analysis:

The results were expressed as the mean ± SD in each group. The data were analyzed with Graph prism statistical software (version 6), San Diego, CA, USA). The differences among the 2 studied groups were examined using t-test. A statistical probability of $P < 0.05$ was considered to be significant and was indicative of the differences in comparison to control.

Results:

Demographic and perinatal characteristics:

Demographic and perinatal characteristics from our study are demonstrated in Table 1. There were no differences in age or weeks of gestation among the groups. The body mass index (BMI) of the PE group was higher than the control group, and the difference was statistically significant. Moreover, systolic and diastolic blood pressure measurements, 24 hs proteinuria, serum creatinine, uric acid and BUN were significantly elevated in the preeclampsia group compared to the gestationally age-matched normotensive control women ($p < 0.0001$), thus confirming the diagnosis of preeclampsia.

Mitofusin2 (Mfn2) mRNA relative expression:

Our data revealed that Mfn2 mRNA expression (assessed by qRT-PCR) in placental tissue of the PE group were significantly downregulated compared to the gestationally age-matched normotensive control group ($p < 0.0001$), suggesting defective mitochondrial fusion. These data are illustrated in Figure 1.

Table 1: *Comparison between the demographic and perinatal characteristics of the both preeclamptic patients and normotensive control groups*

Parameters/Groups	Normotensive control	PE	Unpaired t-test	
	(n=20)	(n=20)	T	P value
Maternal age (years)	25.4±3.9	26.7±4.6	0.96	**> 0.05**
Gestational age (wks)	32.23±4.13	31.49±3.56	0.60	**> 0.05**
BMI (kg/m2)	24.8±2.4	27.8±1.5	4.74	**< 0.0001***
Systolic BP (mmHg)	117.2±11.3	158.6±16.7	9.18	**< 0.0001***
Diastolic BP (mmHg)	74.3±7.6	110.4±8.1	14.54	**< 0.0001***
24 h proteinuria (g)	0.09±0.03	2.12±0.59	15.37	**< 0.0001***
Creatinine (mg/dL)	0.4±0.03	0.85±0.08	23.55	**< 0.0001***
BUN (mg/dL)	5.58±0.95	11.6±1.2	17.59	**< 0.0001***
Uric Acid (mg/dL)	**2.88±1.7**	**5.95±0.78**	**7.34**	**< 0.0001***

Values are expressed as mean ± SD. **P* was considered significant at <0.05. t-test was used for comparison between the 2 groups.

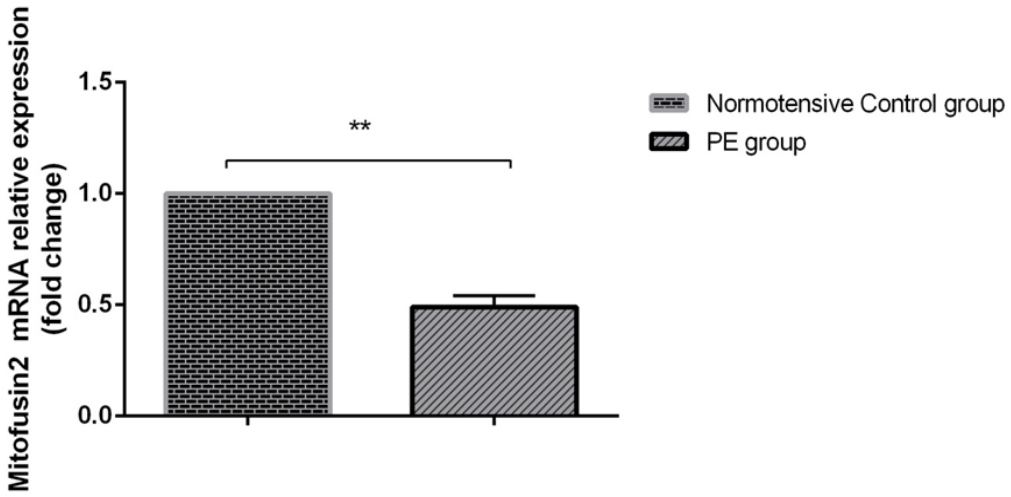

Figure 1. Mfn2 mRNA relative expression levels.
Values are expressed as mean ± SD. ** Significance vs control group using t-test.

Markers of the placental mitochondrial content, energetic function, fission and apoptotic markers:

As demonstrated in table (2), the mitochondrial complex I activity, ATP levels and citrate synthase enzyme activity were assessed as markers of the placental mitochondrial content, energetic function where they were significantly decreased in preeclamptic women compared to the gestationally age-matched normotensive control group ($p < 0.0001$), suggesting mitochondria dysfunction. Meanwhile, the mitochondrial fission protein Drp-1 was significantly elevated in preeclamptic women compared to the gestationally age-matched normotensive control group ($p < 0.0001$), suggesting disturbed mitochondria dynamics with prevailing mitochondrial fission to fusion.

Moreover, our data displayed increased cytosolic cytochrome c levels and raised cytosolic to mitochondrial cytochrome c ratio in preeclamptic women compared to the gestationally age-matched normotensive women ($p < 0.0001$), indicating enhanced release of cytochrome c from the mitochondrial intermembrane space into the cytosol. Likewise, caspase-9 levels were significantly higher in preeclamptic women compared to the gestationally age-matched normotensive control ($p < 0.0001$), signifying activation of the mitochondrial pathway of apoptosis in PE as a consequence to mitochondrial dysfunction.

Redox status parameters:

Our resulted demonstrated significant elevations of the levels NO and 3-NT (as a marker for protein nitration), concomitantly with significant reduction in the total SOD activity in placenta of preeclamptic women compared to the gestationally age-matched normotensive control women ($p < 0.0001$), thus providing an evidence for an altered redox status. These data are summarized in table 3.

Table 2 : *Comparison between biochemical parameters in preeclamptic patients and normotensive controls*

Parameters/Groups	Normotensive control	PE	Unpaired t-test	
	(n=20)	(n=20)	T	P value
Mitochondrial complex I activity (nmol/min/mg protein)	32.04 ±8.52	22.71±4.25	4.38	**< 0.0001***
Mitochondrial ATP levels µmol/g	29.5 ±7.2	14.9 ± 5.1	7.40	**< 0.0001***
Citrate synthase enzyme activity (nmol/min/mg protein)	194.72 ±35.79	147.56 ± 19.06	5.20	**< 0.0001***
Dynamin related protein (Drp1) (ng/mg protein)	129.25±38.61	243.11±44.73	8. 61	**< 0.0001***
Mitochondrial Cyt. C (ng/mg protein)	26.54±4.32	18.12±3.11	7.07	**< 0.0001***
Cytosolic Cyt. C (ng/mg protein)	6.23±1.02	9.87±2.13	6.89	**< 0.0001***
Cyt. C Cytosolic / Mitochondrial	0.24±0.04	0.55±0.07	17.20	**< 0.0001***
Caspase-9 activity (U/mg protein)	**5.04±0.72**	**6.81±1.31**	**5.29**	**< 0.0001***

Values are expressed as mean ± SD. **P* was considered significant at <0.05. t-test was used for comparison between the 2 groups.

Table 3: *Comparison between the redox status parameters in preeclamptic patients and normotensive controls*

Parameters/Groups	Normotensive control	PE	Unpaired t-test	
	(n=20)	(n=20)	T	P value
NO levels (nmol/mg protein)	6.1±1.5	9.2±0.8	8.15	**< 0.0001***
3-Nitrotyrosine (nmol/mg protein)	0.17 ± 0.03	0.34 ± 0.09	8.01	**< 0.0001***
Superoxide dismutase (SOD) activity (U/mg protein)	**14.01±1.47**	**7.35±1.36**	**14.87**	**< 0.0001***

Values are expressed as mean ± SD. **P* was considered significant at <0.05. t-test was used for comparison between the 2 groups.

Discussion:

Preeclampsia (PE) is a pregnancy-specific syndrome, characterized by the novel onset of hypertension and a series of other systematic disorders caused by renal injury (such as proteinuria and edema)[1]. Despite being one of the leading causes of maternal and fetal morbidity and mortality, the etiology and mechanisms responsible for the pathogenesis of PE remain to be elucidated.

Placental mitochondria provide most of the energy production in cells, participate in a number of important cellular processes, and have a central role in placental implantation, growth, and development[6]. Mitochondrial dysfunction has been incriminated in the pathogenesis of PE, although evidence supporting this hypothesis remains inconsistent. Therefore, this study aimed to explore the potential role of placental mitochondria in development of PE and to investigate in depth the contribution of mitochondrial dynamics, biogenesis, redox signaling and apoptosis as potential mechanisms underlying the pathogenesis of mitochondrial dysfunction in the placentas of PE patients.

In this study, we investigated the placental mitochondrial energetic function by measuring the activity of complex I of the electron transport chain (ETC) and cellular ATP levels. Our data revealed a significant decrease in complex I activity in placentas of PE patients compared to normotensive controls. Well in line, Muralimanoharan et al., [22] have reported reduction in the protein expression of complex I in the preeclamptic placentas.

Being the largest and most complex enzyme of the ETC and is a major source of ROS production, complex I is mostly prone to impairment[23]. Local concentrations of ROS near the ETC components are believed to be making the ETC complexes prone to ROS damage. Given that damage to Complex I is particularly important because the mitochondrial inner membrane contains significantly lower complex I than any of the other complexes therefore, damage to a single molecule of this complex would have greater impact on mitochondrial respiration than damage to any of the other complexes[24]. Viewed this way, the reported reduction in complex I activity suggests an altered electron flow through this

complex and possible damage to other complexes, which may contribute to excessive generation of reactive oxygen and nitrogen species and thus mitochondrial dysfunction. Moreover, our data show that ATP levels were significantly decreased in placentas of PE patients compared to normotensive control. These results are essentially consistent with previous studies that have reported that the placenta from PE patients possess a lower ATP level[25]. Two mechanisms may be envisaged for the observed low ATP levels; the first is the altered ATP synthase gene expression in the PE placenta [26] , whilst the second is the heightened state of oxidative stress in PE , resulting from to deficient remodeling of spiral arteries, exposes the PE placenta to fluctuating oxygen concentration and this periodic hypoxia would deplete cellular ATP[27].

Mitochondrial content was further assessed by measuring citrate synthase (CS) activity (as a marker of aerobic capacity and mitochondrial density), where its activity was significantly diminished in placentas of PE patients compared to normotensive control.

In harmony with this finding, Bartha et al. [28]showed that placenta of preeclamptic women exhibited low citrate synthase activity compared to that of normotensive controls. Collectively, these findings validate the theory that mitochondrial dysfunction and disturbed mitochondrial bioenergetics in PE might be partially due to alterations in mitochondrial contents.

Mitochondria are highly dynamic through continuous fusion and fission as well as movement along the cytoskeleton within the cells [29]. Extensive research has led to the consensus that mitochondrial dynamics is an important constituent of cellular quality control, having imperative roles not only in the modulation of mitochondrial morphology but also in other biologic processes, including bioenergetics, cellular metabolism, mitochondrial maintenance, synaptic integrity and neuronal cell death[30].

Mitofusin2 (Mfn2) is an essential component of the mitochondrial quality control system. It is a protein of the outer mitochondrial membrane that promotes membrane fusion and is involved in the maintenance of the mitochondrial network and bioenergetics. An attempt was therefore made to scrutinize the differential expression of the mitochondrial fusion agent Mfn2 between PE placenta and normotensive control ones. Our data revealed that the mRNA expression levels of mfn2 were significantly downregulated in PE placentae compared to normotensive controls.

In agreement with our finding, Yu et al. ,[25] have also reported that Mfn2 expression levels in placenta of PE patients were remarkably lower than normotensive controls. They also demonstrated that Mfn2 knockdown led to decreased viability of trophoblastic cells and thus concluded that Mfn2 may be functionally involved in the pathogenesis of PE through causing mitochondrial dysfunction and thus decreased viability of trophoblastic cells. One hypothesis to explain this effect may be based on the fact that the mitochondrial fusion provides a synchronized internal cable for the exchange of intramitochondrial contents including mtDNA to complement damaged contents, fusion-deficient mitochondria have a defect in oxidative phosphorylation as they are unable to restore

and/or maintain the mtDNA-encoded proteins required for electron transport[31]. This notion is further corroborated by results of recent studies demonstrating that Mfn2 deficiency promotes mitochondrial outer membrane injury and thus contributes to mitochondrial dysfunction, fragmentation and enhanced cell death in pulmonary arterial hypertension [10] and renal stress response[32].

On the other hand, mitochondrial fission in eukaryotic cells depends on dynamin-related protein 1 (Drp1), a cytoplasmic dynamin GTPase that participates in the fragmentation of mitochondria, peroxisomes and ER[33]. In the present study, a DRP1 mitochondrial protein levels were significantly elevated in PE placentae compared to normotensive controls. This finding is biologically plausible as Drp1 primarily exists in the cytosol as a dimer/tetramer and translocates from the cytosol to mitochondria during mitochondrial fission, where it oligomerizes around and constricts the mitochondria, thereby leading to severing of the mitochondrial membrane by GTP hydrolysis[34]. Accordingly, the reported elevation in mitochondrial Drp1 protein levels in PE could be attributed to placental hyoxia which is known to facilitate Drp1 translocation to mitochondria and thus enhances mitochondrial fragmentation[35]. Different from our observation, Vishnyakova et al. [36] reported no difference in DRP1 expression levels between preeclamptic and control placentas. These apparently inconsistent findings might be decoded on the basis of possible accelerated Drp1 translocation to mitochondria in the presence of a constant transcription rate.

Taken together, the data obtained in this study begin to outline an overarching role of altered mitochondrial dynamics, with prevailing mitochondrial fission to fusion, as part of the molecular mechanisms underlying mitochondrial dysfunction in PE. It has been widely reported that mitochondria are also crucial targets of ROS that can modulate the function of this organelle under physiological and pathological conditions[37].

Therefore, we investigated whether ROS generation contributes to mitochondrial dysfunction in PE. In this sense, our study provided an evidence for altered redox status as judged by the significant elevation of NO and protein nitration concomitantly with significant reduction in the total SOD activity in placenta of PE patients compared to normotensive controls. These results are not without precedence, being previously noted by Vanderlelie et al., [38] who demonstrated a decreased levels of SOD , the master enzyme in the regulation of cellular redox status, along with increased lipid and protein oxidation in placental tissue from pre-eclamptic women, which may contribute to the pathogenesis of this complex disorder.

Meanwhile, our findings concur with the reported increased placental NO production in pre-eclampsia than those of normal pregnancy; this increase was directly related to the severity of this disorder, representing a physiologic adaptive response to overcome the increased placental vascular resistance[39]. Regarding protein nitration which is a reliable marker for in vivo biological nitration and NO dependent damage, Chekir et al., [40] observed that the intensity of immunostaining of nitrotyrosine in the placentas

from preeclamptic women was greater than that in the placentas from healthy women, lending credence to our findings .

Taken in conjunction, it is conceivable that when superoxide and NO are present in abundance, their interaction yields peroxynitrite which reacts with proteins forming nitrotyrosine [41]. Several hypotheses invoke oxidative / nitrative stress as a cellular process contributing to altered placental mitochondrial function in PE; possibly via mutations of mtDNA and inhibition of ETC components as a result of cysteine oxidation, tyrosine nitration and damage of iron sulfur centers; which in turn contributes to altered energy metabolism and increased apoptosis[15]. The increased ROS/RNS production triggers a vicious cycle of exponentially increasing levels of mtDNA damage and oxidative/nitrosative stress in the cell over time [42].

Mitochondria play a crucial role in the regulation of apoptosis[13]. Consistent with this, our results displayed activation of the mitochondrial pathway of apoptosis as judged release of cytochrome c from the mitochondrial intermembrane space into the cytosol and significant increase in caspase-9 levels in placentas of PE compared to that of normotensive controls.

An intriguing possibility to this perturbated apoptotic cell death could be the observed Mfn2 downregulation, along with excessive mitochondrial fission mediated by Drp1 which would produce dysfunctional mitochondrial fragments that show increased ROS production and formation of mitochondrial pores with release of intermembrane space proteins that can either act as cofactors for the assembly of the Apaf-1/caspase-9 apoptosome or promote other downstream events in apoptosis, ultimately leading to caspase-3 activation and subsequent cell death [43].

Our results came in accordance with a previous study reported that Mfn2 plays a decisive role in successful preimplantation development and its inadequate expression affects embryonic development through mitochondrial dysfunction and induction of apoptosis [44].

In conclusion, the present study contributed information towards establishing a significant association between mitochondrial dysfunction, disturbed dynamics, altered redox status and the susceptibility to apoptosis in preeclamptic placenta. Our data strongly nominated these biological processes as key players in the multifactorial pathogenic mechanisms of PE and as potential therapeutic targets. Future research is warranted to dissect the complex crosstalk between mitochondrial dynamics and proper cell functions and to provide exciting breakthroughs in the fields of PE treatment and prevention.

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