https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(4s) (November 2024); 13117-13123
Research Article

Elevated Serum sFlt-1 In Preeclamptic Mothers Activates GRP78 In Placenta

Sankat Mochan[1*], Chandan Kumar Pal[2], Rinke Choudhary[3], Vishnu Swarup[4], Lakshay Malhotra[5] , Mohd Anas Khan[6], Pallavi Arora[7], Renu Dhingra[8]

[1*]Department of Anatomy, University College of Medical Sciences and GTB hospital, Delhi, India
[2]Department of Radio Diagnosis, University College of Medical Sciences and GTB Hospital, Delhi, India
[3]Department of Anatomy, University College of Medical Sciences and GTB hospital, Delhi, India
[4]Department of Neurology, All India Institute of medical Sciences, New Delhi, India
[5]Department of Biochemistry, Sri Venkateswara College, University of Delhi, New Delhi, Delhi, India
[6]Department of Anatomy, Hamdard Institute of Medical Sciences and Research, New Delhi, India
[7]Department of Anatomy, Maharishi Markandeshwar Institute of Medical Sciences & Research, Mullana, Ambala, India
[8]Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India

***Corresponding Author:** Dr. Sankat Mochan
Email: smochan@ucms.ac.in

Abstract
Objective: To find out the role of sFlt-1(sVEGFR1) in the induction of the GRP78 in pregnant women with preeclampsia
Methods: The study was divided into two parts
(1) Serum Analysis of sFlt-1, and GRP78 was done using ELISA.
(2) In Vitro experiments: Activation of Endoplasmic reticulum (ER) stress marker GRP78 was assessed at various time points (8h, 14h, 24h) at protein level (Immunofluorescence).
Results: Raised levels of a central regulator of unfolded protein response, GRP78, and sFlt-1 were reported in preeclamptic sera. The expression of GRP78 in BeWo cells (treated with sera from preeclamptic and normotensive, nonproteinuric women) at protein level were compared at three different time points, analyzed and found to be significant
Conclusion: Significant expression of GRP78 was reported in placental cells (BeWo Cells) (*in vitro*) when exposed to sera from preeclamptic pregnancies with an increased concentration of sFlt-1.
Implication: Results suggested that sFlt-1 released from placental cells in preeclampsia, may be one of the various factors with the potential to activate GRP78 and thus induce endoplasmic reticulum stress in BeWo cells, which mimic placental cells.

Author of correspondence: Email: *smochan@ucms.ac.in*

Received:10/10/2024 *Accepted:20/10/2024*

DOI: https://doi.org/10.53555/AJBR.v27i4S.6646

Introduction

sFlt-1, a splice variant of VEGF receptor Flt-1, lacks the transmembrane and cytoplasmic domains, is made in large amounts by placental trophoblasts, and is released into maternal circulation[1,2]. It acts as a potent anti-angiogenic molecule by binding circulating VEGF and PlGF. Several studies worldwide reported that the sFlt-1 gene product is up-regulated in the preeclamptic placentae[3,4,5]. Alterations in the angiogenic molecules (increased sFlt-1, decreased free VEGF and PlGF) antedate the onset of clinical symptoms of preeclampsia (PE) which is generally defined as new hypertension (diastolic blood pressure of >90 mm Hg or systolic blood pressure of >140mm Hg) and substantial proteinuria (≥300 mg in 24 h) at or after 20 weeks of gestation[6,7]. It is a common disorder (5-8% of all pregnancies) and often requires premature delivery of the baby[8,9]. Occasionally, PE may lead to seizures and multi-organ dysfunction in the mother with severe morbidity and even mortality[10]. It occurs only in the presence of the placenta and remits dramatically postpartum after the placenta has been delivered[11]. The placentae of preeclamptic women appear wayward, with evidence of underperfusion and ischemia[12]. The initial event in PE has been posited to be reduced uteroplacental perfusion which develops as a result of abnormal cytotrophoblast invasion of spiral arterioles, triggering the cascade of events leading to the maternal disorder[13]. Such malperfusion leads to poor placentation which causes both oxidative and endoplasmic reticulum stress in the placenta[14]. The Endoplasmic reticulum (ER) performs multiple functions, including the synthesis, post-translational modification, and trafficking of membrane and secreted proteins[15]. Fuss of ER homeostasis may result in misfolding or abnormal glycosylation of these proteins, which may reduce their biological activity[16]. The accumulation of unfolded or misfolded proteins within the ER cisternae incites ER stress and activation of the unfolded protein response (UPR) [17]. The UPR ventures to restore ER function by attenuating protein translation, increasing folding capacity, and facilitating the mortification of misfolded proteins[18]. It consists of three leading signaling pathways with overlapping functions[19]. The three trans-membrane sensors of UPR consist of PKR-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring 1 (Ire1), project into the ER lumen[20]. The present study was designed to explore the effect of sFlt-1 on the induction of GRP78 and thus in the activation of the endoplasmic reticulum stress pathway (*in vitro* study). We demonstrated ER stress in BeWo cells (human choriocarcinoma cell line of placental origin) because these cells mimic *in vivo* syncytialization of placental villous trophoblast.

Materials and Methods

Study Subjects:

60 pregnant women were enrolled in the antenatal clinic and the inpatient ward of the Department of Obstetrics and Gynaecology, All India Institute of Medical Science, New Delhi, India. The preeclamptic women (n=30) after clinical diagnosis were enrolled as cases and normotensive, non-proteinuric pregnant women (n=30) (maternal and gestational age-matched) without any other medical complications were enrolled as controls. Protocol of the study was approved by the Institute Ethics Committee, and written informed consent was obtained from all the enrolled women. Preeclampsia was defined according to ACOG guidelines: Blood Pressure- 140 mm Hg systolic or >90mm Hg diastolic on 2 occasions at least 4 hours apart after 20 weeks of gestational age in women with a previously normal BP and 160 mm Hg systolic or >110 mm Hg diastolic, confirmed within a short interval (minutes) to facilitate timely antihypertensive therapy; Proteinuria >300 mg per 24-hour urine collection or protein/ creatinine ratio > 0.3mg/dl or dipstick reading of >1+ or in the absence of proteinuria, new-onset hypertension with new onset of one or more of the following thrombocytopenia: platelet count <100,000/μl, renal insufficiency: serum creatinine > 1.1mg/dl or doubling of serum creatinine in the absence of other renal disease, impaired liver function: elevated blood levels of liver transaminases to twice normal concentrations, pulmonary edema and cerebral edema. Pregnant women with chronic hypertension, chorioamnionitis**,** diabetes**,** renal disease**,** and cardiac disease were excluded from the study. 5 ml of venous blood was collected, and centrifuged at 1200 RPM for 4 minutes, and serum was separated and stored in aliquots at -80˚C. The serum samples were stored for the ELISA and cell culture experiments.

Serum analysis of sFlt-1 and GRP78 using ELISA:

The levels of sFlt-1 and GRP78 were estimated in the serum of preeclamptic and normotensive, non-proteinuric pregnant women groups by sandwich ELISA (sFlt-1 ELISA kit: R&D Systems Inc., Minneapolis, MN, U.S.A., GRP78: Enzo Life Sciences, Inc.).

***In vitro* experiments** were carried out to analyze the effect of sFlt1-1 on the induction of ER stress in trophoblastic cells (BeWo cells). The human choriocarcinoma cell line (BeWo) was procured from American Type Culture Collection (ATCC) and maintained in F-12 HAM nutrient medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100μg/ml Streptomycin. Cells were passaged with 0.025% trypsin and 0.01% EDTA.

The study was divided into two experimental groups depending on different treatments given to BeWo cells: group 1 [normotensive, non-proteinuric serum (low concentration of sFlt-1)], group 2 [preeclamptic serum (high concentration of sFlt-1)]. After the treatments, activation of GRP78 was assessed at various time points at the protein level (Immunofluorescence).

Immunofluorescence Microscopy:

BeWo Cells were trypsinized, seeded, allowed to grow on coverslips in multiple well chambers, and incubated at 37℃ in 5% CO2. After 8h, 14 h, and 24 h, cells were taken out from the incubator and washed with PBS, fixed in 4% PFA for 15 min at room temperature. After fixation, cells were washed with PBS and

permeabilized with PBS + 0.1% Triton X-100 followed by PBS washing. Nonspecific blocking was done using 5% normal goat serum in PBS and Triton X. Cells were incubated for 12 hours at 4℃ in primary antibodies (anti GRP78 antibody (1:1000). Cells were washed with PBSTx and thereafter incubated in secondary antibody in 1:500 dilution for 1 hour at room temperature in a dark room. Cells were washed in PBS and mounted with flourished mounting media with DAPI on the slide and observed under the fluorescence microscope (Nikon Eclipse Ti-S elements using NiS-AR software).
All methods were performed according to the relevant guidelines and regulations.

Statistical Analysis

Data was analyzed by Microsoft Office Excel Version 2013 and Graph Pad Prism. The average level of the variable between the two groups was compared by paired t-test. *p* value<0.05 was considered statistically significant.

Results

The maternal serum of 60 pregnant women was analyzed. The mean systolic and diastolic blood pressures in the preeclamptic group were 158.9 ± 11.88 mm Hg (mean ± SD) and 101.43 ± 8.39 mm Hg (mean ± SD) respectively whereas in the control group, the systolic and diastolic pressures were 117.8 ± 7.34 mm Hg (mean ± SD) and 74.2 ± 6.39 mm Hg (mean ± SD) respectively. The difference in the systolic and diastolic pressures between the groups was statistically significant (p<0.0001). The body mass index in preeclamptic patients was 27.83± 5.61 (mean ± SD) and in the control group was 23.67 ± 3.59 (mean ± SD). The difference was statistically significant (p<0.0001). Urine protein in preeclamptic and control group women was analyzed by urine dipstick method. 10% of preeclamptic women (3/30) showed 1+ urine protein, 43.33% showed 2+ urine protein (13/30) and 46.66% showed 3+ urine protein (14/30) (Table 1)

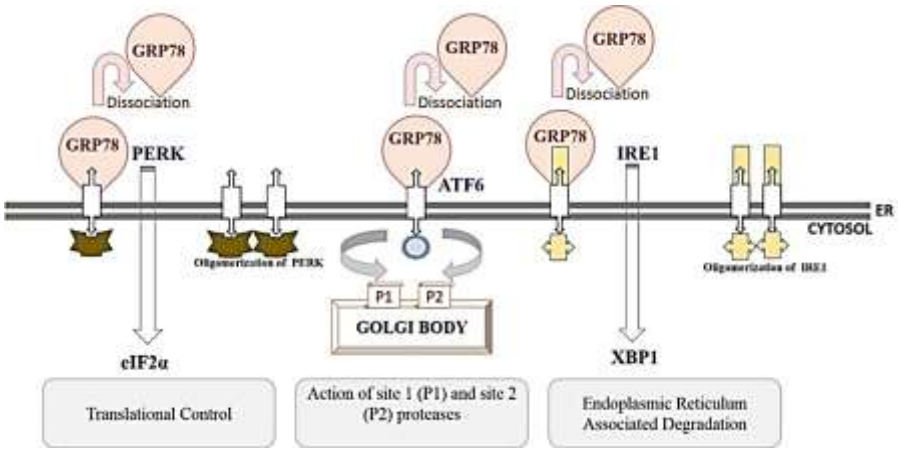

Figure 1. Dissociation of GRP78 from trans-membrane sensors and initiation of Endoplasmic Reticulum stress pathway

Table 1: Clinical Characteristics of Preeclamptic and normotensive, non proteinuric pregnant women (controls). n= number of subjects, Data presented as mean ± SD, Paired t test, *statistical significance, p<0.05

Study Groups			
Clinical characteristics	Preeclampsia (n=30)	Normotensive, Non proteinuric (Control) (n=30)	Statistical significance (p value)*
Systolic blood pressure (mmHg)	158.9 ±11.88	117.8±7.34	p<0.0001
Diastolic blood pressure (mmHg)	101.43±8.39	74.2±6.39	p<0.0001
Body Mass Index	27.83±5.61	23.67±3.59	p<0.0001
Protein (gm/day)	4.9±1.6	0.7±0.2	p<0.0001

Elevated levels of salt-1 and GRP-78 were observed in preeclamptic patient's sera:

The level of sFlt-1 in the maternal serum of preeclamptic patients was 11295.25 (2936.2-37818) pg/ml and in the control group was 2936.2 (1180.43-6706.6) pg/ml. The serum sFlt-1 levels were significantly higher in women with preeclampsia than in the control group and the difference was statistically significant. (p=0.0001) (Table 2, Figure 2). The level of GRP78 in the maternal serum of preeclamptic patients was 1103.26 + 104.27 ng/ml and in the control group was 1018.61 + 125.51 ng/ml. The serum GRP 78 levels were higher in the sera of women with preeclampsia than in the control group and the difference was statistically significant. (p=0.012) (Table 2, Figure 3).

Table 2: Maternal serum levels of sFlt-1 and GRP78 in preeclamptic and normotensive, non proteinuric pregnant women (controls). n= number of subjects, Data presented as median (range), Paired t test, *statistical significance, $p<0.05$

Serum levels	Preeclampsia n=30	Normotensive non proteinuric (Control) n=30	Statistical significance (p Value)*
sFlt-1	11295.25 2936.2 - 37818 Median (Range)	2936.2 1180.43 - 6706.6 Median (Range)	0.0001
GRP78	1103 ± 104.27 Mean±SD	1018.61 ± 125.51 Mean±SD	0.012

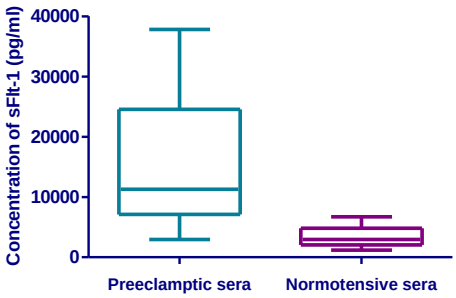

Figure 2: Box plots showing serum levels of soluble fms-like tyrosine kinase-1 (sFlt-1). Boxes denote the interquartile range with the upper and lower horizontal edges representing the 75th and 25th percentiles. The central horizontal lines represent the medians. *Statistical significance, $p<0.05$

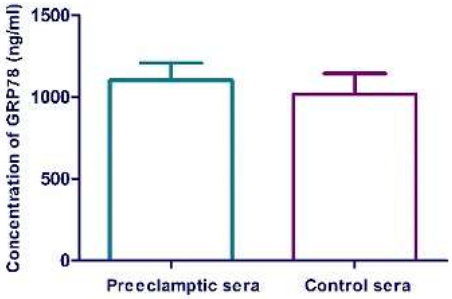

Figure 3: Bar diagram represents serum levels of Glucose Regulated Protein78 (GRP78). Values are represented as Mean +SD. Error bars represent standard deviation. *Statistical significance, $p<0.05$

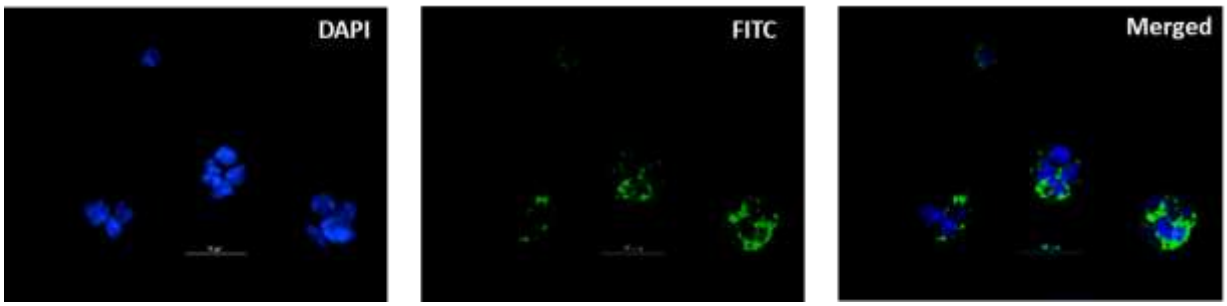

Figure 4: Representative immunofluorescence staining pattern (weak expression) of anti-GRP78 antibody positive BeWo cells following treatment with normotensive sera (group 1) (low sFlt-1 concentration)

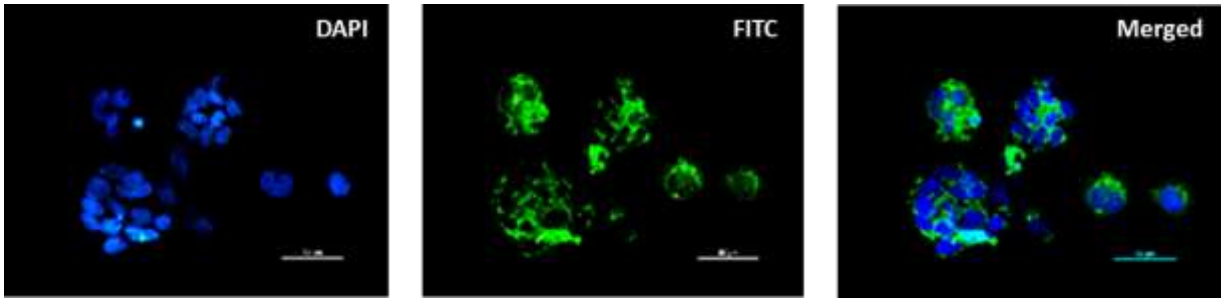

Figure 5: Representative immunofluorescence staining pattern of anti-GRP78 antibody positive BeWo cells following treatment with preeclamptic sera (group 2) (high sFlt-1 concentration).

***In Vitro* Experiments:**

Weak expression of GRP78 was observed in BeWo cells treated with sera from Normotensive (NT) pregnant women [Figure 4]: Immunofluorescence microscopy revealed weak significant expression of GRP78.

Significantly increased expression of GRP78 was observed in BeWo cells treated with Preeclamptic sera (PE) [Figure 5]: Immunofluorescence microscopy revealed significant expression of GRP78.

Discussion

In the present study, we reported an increased level of sFlt-1 in women with preeclampsia suggesting an anti-angiogenic state may be involved in the pathogenesis of PE[21]. Angiogenesis, the formation of new blood vessels from pre-existing ones, is essential for a successful pregnancy. The role of VEGF as a key regulator of normal and abnormal angiogenesis has already been established over the years[22]. Under normal physiological conditions, VEGF exerts its biological effects through two high affinity tyrosine kinase receptors VEGFR1 or fms like protein kinase-1/Flt-1 and VEGFR2 or kinase insert domain receptor/KDR[26,27]. The soluble isoform of VEGFR1 (Flt-1) known as s-VEGFR1(sFlt-1), binds with free VEGF in circulation with higher affinity thereby preventing its interaction with its endogenous receptors (VEGFR1 and VEGFR2) and thus reducing its biological effects like placental development and maintenance of maternal vasculature. Thus, the reduced free VEGF due to increased sFlt-1 (imbalance of pro and anti-angiogenic factors) could alter the balance of superoxide anion and nitric oxide, resulting in increased oxidative stress contributing to vascular endothelial dysfunction[28]**.** Although it is known that both stress processes (oxidative stress and ER stress) are intimately related, the mechanisms linking them are not fully understood, however, these two may be linked in three principal ways[29]. Placental endoplasmic reticulum stress has recently been recognized in the pathophysiology of preeclampsia. The vulnerability of placental cells i.e., syncytiotrophoblast to ER stress may be due to their involvement in extensive secretory activity. Also, molecular and morphological evidence confirms high levels of ER stress in placentas from cases of early onset of preeclampsia as compared to late onset[30].

After the onset of ER stress (on the failure of proteostasis), the initial event is the dissociation of GRP78 from trans-membrane sensors, PERK (PKR-like ER kinase), IRE1 (inositol-requiring enzyme 1) and ATF6 (activated transcription factor 6). These sensors are integral ER membrane proteins that signal luminal stress to the cytosol and the nucleus. When ER stress is not mitigated and homeostasis is not restored, the UPR (unfolded protein response) triggers apoptosis. The mammalian UPR, in the chronological time frame, has evolved into a network of signaling events that responds to various inputs over a wide range of basal metabolic states. Several conditions can activate the UPR, including changes in glycosylation, redox status, glucose availability, calcium homeostasis, and secretory protein load[31]. Thus, in the present study, we hypothesized that levels of GRP78 are raised in sera of patients of PE. To validate this hypothesis, we estimated the GRP78 levels in serum by sandwich ELISA. The maternal serum levels of GRP78 were found to be higher in PE patients as compared to normotensive, non-proteinuric pregnant women (Table 2, Figure 3).

With this finding, we deciphered that the raised levels of GRP78 in patients' sera might be the reason for its earlier dissociation from trans-membrane sensors and its subsequent release in the maternal serum thereby inciting ER stress following signaling through individual ER stress arms. However, these results did not establish a cause-and-effect relationship i.e. whether the sFlt-1 is the causative factor for increased GRP78 found in PE patients. To answer this question, our group for the first time performed *in-vitro* experiments using BeWo cells which received various treatments having varying concentrations of sFlt-1 (normotensive and preeclamptic sera), and the expression of ER stress markers were analysed at transcript and protein levels at different time points. The three different time points of study (8h, 14h, and 24 h) were chosen to observe the activation and expression of the marker after activation of the ER stress pathway (confirmed after the signal from the master regulator of UPR).

The prime regulator of UPR i.e., GRP78, in the resting condition, binds to the N-terminus of trans-membrane sensors, but it's dissociation and subsequent competitive binding to accumulating misfolded proteins leads to dimerization, autophosphorylation, and activation of PERK and IRE1. The expression of GRP78 protein was detected as early as 8h however

maximum expression varied from 14 to 24 h in various groups.

The level of GRP78 protein was further upregulated when BeWo cells were treated with sera having high sFlt-1 concentration (PE sera) [figure 5]. The upregulated expression of GRP78 thus suggested the presence of a common factor that might increase the expression of GRP78 at the protein level. We inferred this factor might be sFlt-1.

Emerging evidence indicates amplitude and kinetics of UPR signaling are tightly regulated at different levels, which has a direct impact on cell fate decisions. How protein folding stress at the ER is sensed has been a central topic in the research field for more than a decade. Different models have been proposed to explain how ER stress is sensed, and these are constantly modified over time owing to new findings and discrepancies and similarities between the yeast and mammalian UPR[32]. In the present study, we focused on the effects of sFlt-1 on GRP78 or in other words, the initiation of the ER stress pathway and found that the marker of ER stress was up-regulated though at different time points when BeWo cells were treated with high sFlt-1 concentration and their expression reduced on treatment with low sFlt-1 concentration.

Summary and conclusion

In the present study, we demonstrated increased expression of GRP78 in trophoblast cells (BeWo cells) which reasserted the setback of unfolded protein response aiming to maintain proteostasis of the cellular environment. The increased signaling response of ER stress makers in the BeWo cells exposed to increased sFlt-1 concentration gesticulated that sFlt-1 may be one of the various factor(s) responsible for endoplasmic reticulum stress seen in placental trophoblast cells of preeclamptic women. The raised serum GRP78 levels observed in women with preeclampsia as compared to normotensive non-proteinuric pregnant women might be due to raised sFlt-1. Our study can serve as an experimental and therapeutic template to investigate newer therapeutic regime in the management of preeclampsia.

Conflict of Interest

None

References

1. Banks, R.E., Forbes, M.A., Searles, J., Pappin, D., Canas, B., Rahman, D., Kaufmann, S., Walters, C.E., Jackson, A., Eves, P., Linton, G., Keen, J., Walker, J.J., Selby, P.J. Evidence for the existence of a novel pregnancy-associated soluble variant of the vascular endothelial growth factor receptor, Flt-1. Mol Hum Reprod., 4(377), pp. 386 (1998).
2. Clark, D.E., Smith, S.K., He, Y., Day, K.A., Licence, D.R., Corps, A.N., Lammoglia, R., Charnock-Jones, D.S. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. Biol Reprod., 59, pp. 1540–1548 (1998).
3. Tsatsaris, V., Goffin, F., Munaut, C., Brichant, J.F., Pignon, M.R., Noel, A., Schaaps, J.P., Cabrol, D., Frankenne, F., Foidart, J.M. Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. J Clin Endocrinol Metab., 88, pp. 5555–5563 (2003).
4. Maynard, S.E., Min, J.Y., Merchan, J., Lim, K.H., Li, J., Mondal, S., Libermann,T.A., Morgan, J.P., Sellke, F.W., Stillman, I.E., Epstein, F.H., Sukhatme, V.P., Karumanchi, S.A. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest., 111, pp. 649–658 (2003).
5. Zhou, Y., McMaster, M., Woo, K., Janatpour, M., Perry, J., Karpanen, T., Alitalo, K., Damsky, C., Fisher, S.J. Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome., Am J Pathol, 160, pp. 1405–1423 (2002).
6. Levine, R.J., Maynard, S.E., Qian, C., Lim, K.H., England, L.J., Yu, K.F., Schisterman, E.F., Thadhani, R., Sachs, B.P., Epstein, F.H., Sibai, B.M., Sukhatme, V.P., Karumanchi, S.A. Circulating angiogenic factors and the risk of preeclampsia., N Engl J Med, 350, pp. 672–683 (2004).
7. Tannetta, D., Sargent I. Placental Disease and the Maternal Syndrome of Preeclampsia: Missing Links?., Current Hypertension Reports, 15(6), pp. 590–599 (2013).
8. Svein, R., Ebbing, C., Irgens, L.M. Predicting Preeclampsia from a History of Preterm Birth., PLOS ONE, 12(7), pp. e0181016 (2017).
9. Pennington, K.A., Schlitt, J.M., Jackson, D.L., Schulz, L.C., Schust, D.J. Preeclampsia: multiple approaches for a multifactorial disease., Disease Models & Mechanisms, 5(1), pp. 9-18 (2012).
10. Cipolla, M.J., Kraig, R.P. Seizures in Women with Preeclampsia: Mechanisms and Management., Fetal and maternal medicine review, 22(2), pp. 91-108 (2011).
11. Cerdeira, A.S., Karumanchi, S.A. Angiogenic Factors in Preeclampsia and Related Disorders., Cold Spring Harbor Perspectives in Medicine, 2(11), pp. a006585 (2012).
12. Ogge, G., Chaiworapongsa, T., Romero, R. Placental lesions associated with maternal underperfusion are more frequent in early-onset than in late-onset preeclampsia., Journal of perinatal medicine, 39(6), pp. 641-652 (2011).
13. Palei, A.C., Spradley, F.T., Warrington, J.P., George, E.M., Granger, J.P. Pathophysiology of Hypertension in Preeclampsia: A Lesson in Integrative Physiology., Acta physiologica, 208(3), pp. 224-233 (2013).
14. Burton, G.J., Yung, H.W., Cindrova-Davies, T., Charnock-Jones, D.S. Placental Endoplasmic Reticulum Stress and Oxidative Stress in the Pathophysiology of Unexplained Intrauterine

Growth Restriction and Early Onset Preeclampsia., Placenta, 30, pp. 43-48 (2009).
15. Cooper, G.M. The Cell: A Molecular Approach. 2nd edition., Sunderland (MA): Sinauer associates (2000).
16. Yung, H.W., Hemberger, M., Watson, E.D. Endoplasmic reticulum stress disrupts placental morphogenesis: implications for human intrauterine growth restriction., The Journal of Pathology, 228(4), pp. 554-564 (2012).
17. Stewart, S.C. Endoplasmic reticulum stress and unfolded protein response in inflammatory bowel disease., Inflammatory Bowel Diseases, 21(3), pp. 636–644 (2015).
18. Chen X, Shi C, He M, Xiong S, Xia X. Endoplasmic reticulum stress: molecular mechanism and therapeutic targets. Signal Transduct Target Ther. 2023 Sep 15;8(1):352. doi: 10.1038/s41392-023-01570-w. PMID: 37709773; PMCID: PMC10502142.
19. Jinhua, F., Zhao, L., Wang, L., Zhu, X. Expression of markers of endoplasmic reticulum stress-induced apoptosis in the placenta of women with early and late onset severe pre-eclampsia., Taiwanese Journal of Obstetrics and Gynecology, 54(1), pp. 19-23 (2015).
20. Mukherjee, I., Dhar, R., Singh, S. et al. Oxidative stress-induced impairment of trophoblast function causes preeclampsia through the unfolded protein response pathway. Sci Rep 11, 18415 (2021). https://doi.org/10.1038/s41598-021-97799-y
21. Maynard, S.E., Min, J.Y., Merchan, J., Lim, K.H., Li, J., Mondal, S., Libermann, T.A., Morgan, J.P., Sellke, F.W., Stillman, I.E. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia., J Clin Invest, 111, pp. 649–658 (2003).
22. Napoleone, F. Role of Vascular Endothelial Growth Factor in Regulation of Physiological Angiogenesis., American Journal of Physiology-Cell Physiology, 280(6), pp. C1358-C66 (2001).
23. Jelkman, W. Pitfalls in the measurement of circulating Vascular Endothelial Growth Factor., Clin Chem, 47, pp. 617-23 (2001).
24. Chaiworapongsa, T., Romero, R., Espinoza, J., Bujold, E., Mee Kim, Y., Goncalves, L.F., Gomez, R., Edwin, S. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia., Am J Obstet Gynecol, 190, pp. 1541–1547 (2004).
25. Bridges, J.P., Gilbert, J.S., Colson, D., Gilbert, S.A., Dukes, M.P., Ryan, M.J., Granger, J.P. Oxidative stress contributes to soluble fms-like tyrosine kinase-1 induced vascular dysfunction in pregnant rats., Am J Hypertens, 22(5), pp. 564-8 (2009).
26. Halliwell, B., Gutteridge, J.M.C., Antioxidant defence mechanisms: From the beginning to the end (of the beginning)., Free Radic Res, 31, pp. 261-272 (1999).
27. Burton, G.J., Yung, H.W. Endoplasmic reticulum stress in the pathogenesis of early-onset pre-eclampsia., Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health, 1(1), pp. 72-78 (2011).
28. Zhang, K., Kaufman, R.J. Signaling the unfolded protein response from the endoplasmic reticulum., The Journal of Biological Chemistry, 279(25), pp. 25935–25938 (2004).
29. Kimata, Y., Kohno, K. Endoplasmic reticulum stress-sensing mechanisms in yeast and mammalian cells., Curr. Opin. Cell Biol, 23, pp. 135–142 (2011).
30. Mochan, S., Dhingra, MK., Gupta, SK., Saxena, S., Arora, P., Yadav, V., Rani, N., Luthra, K., Dwivedi, S., Bhatla, N., Dhingra, R. Status of VEGF in preeclampsia and its effect on endoplasmic reticulum stress in placental trophoblast cells. European Journal of Obstetrics & Gynecology and Reproductive Biology X 2019 Jun 21; 4:100070.
31. Woehlbier, U., Hetz, C. Modulating stress responses by the UPRosome: a matter of life and death., Trends Biochem. Sci, 36, pp. 329–337 (2011).
32. Mochan, S., Dhingra, MK., Gupta, SK., Saxena, S., Arora, P., Rani, R., Bhatla, N., Dwivedi, SN., Dhingra, R. Vascular endothelial growth factor alleviates endoplasmic reticulum stress via PERK, IRE1, and ATF6 pathways in trophoblast cells. Journal of Anatomical Society of India 2020 Jan; 68:187-95